Development and feasibility of an electrochemical-oxidation process for water disinfection

by

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Declaration

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Abstract

Quality of water supplies for potable use has deteriorated to such a state that point-of-use water disinfection has become a necessity. Historically, chlorine has been the most widely used disinfectant, but its shortcomings have led to the development of numerous alternative technologies over the last three decades. One of the emerging, but less understood technologies, are metal ions combined with an oxidising agent. Traditionally, chlorine disinfection has been controlled by measuring free chlorine residual and pH, but oxidation reduction potential (ORP) is emerging as an alternative indicator of the efficacy of disinfection.

The objectives of the research were to (i) identify the contribution, if any, of metallic ions on the disinfection ability of bromo-chloro-dimethyl-hydantoin (BCDMH), (ii) investigate the feasibility of using metallic ions with BCDMH as a disinfectant on a typical potable water supply, (iii) evaluate ORP as an indicator of disinfection efficacy for a disinfection process that combines metallic ions with BCDMH. The feasibility investigated criteria such as efficiency, ease of implementation, financial implications and environmental implications, comparing the combined technology with the individual processes.

A batch experimental setup was developed that treated a feed with a combination of metal ions and an oxidising agent. The feed consisted of tap water artificially contaminated with the bacterium *Pseudomonas sp. strain CT07* at a concentration of between $0.5x10^7$ and $2.0x10^7$ cfu/ml. The drop plate method was used to determine disinfection by the reduction of bacterial concentrations to below detection limits. Copper, silver, and zinc ions were released by an ioniser and a BCDMH stock solution was used as oxidising agent. A fixed contact time of 5-minutes was used to keep findings relevant to point-of-use water disinfection. Experimental results were analysed using logistic regression.

The logit model for combined treatment had a strong correlation with a Cox-Snell R^2 of 0.516 and was significant with a p-value <0.001. The interaction coefficient (β_{12}) was significant with a p-value of 0.036. The significant interaction coefficient showed that metal ions could improve the disinfecting ability of BCDMH at a short contact time of 5-minutes. The addition of metallic ions decreased the amount of BCDMH required to attain a certain probability for successful disinfection according to the probability model.

The combined disinfection process is more efficient than the individual processes, can easily be implemented, and has environmental benefits over chlorine treatment. However, the combined technology is more expensive to operate than only BCDMH treatment. The addition of metal ions

through ionisation can lead to a 25.67% reduction in BCDMH used, but it comes at an operating cost 2.5 times higher than treatment without metal ions.

Final ORP values had no correlation with disinfection success. The change in ORP for the BCDMH treatment showed a significant relationship with treatment success with a p-value of 0.018. The relationship showed that a ΔORP of 164.35 mV should correspond to a 90% probability for successful treatment. However, the maximum experimental ΔORP was only 117 mV.

The research showed that metal ions improve the disinfection efficiency of BCDMH. The technology has several advantages, but is not financially justifiable due to the increase in cost when compared to standard BCDMH treatment. The change in ORP was more closely related to disinfection success than to a single ORP value. A full concentration-contact time (CT) investigation would broaden the understanding of the interactions between metal ions, BCDMH, and pathogens.

Uittreksel

Watergehalte van voorsiende water wat vir drink doeleindes gebruik word, het tot so ’n toestand verswak dat punt-van-gebruik waterdisinfeksie ’n noodsaaklikheid geword het. Histories, is kloor die mees gebruikte ontsmettingsmiddel, maar die tekortkominge daarvan het die afgelope drie dekades tot die ontwikkeling van talle alternatiewe tegnologieë gelei. Een van die opkomende, maar minder begrypte tegnologieë is die kombinasie van metaalione met ’n oksideringsagent. Tradisioneel is kloordisinfeksie beheer deur die vrye kloorresidu en die pH te meet, maar oksidasiereduksiepotensiaal (ORP) is besig om as ’n alternatiewe indikator van die doeltreffendheid van ontsmetting na vore te kom.

Die doelwitte van die navorsing was om (i) die bydrae, indien enige, van metaalione op die ontsmettingsvermoë van broom-kloriedimethylhidantoïen (BCDMH) te identifiseer, (ii) die uitvoerbaarheid van die gebruik van metaalione met BCDMH as ’n ontsmettingsmiddel op ’n tipiese drinkwaterbron te ondersoek, (iii) die ORP as ’n indikator van ontsmettingsdoeltreffendheid te evalueer vir ’n ontsmettingsproses wat metaalione met BCDMH kombineer. Die uitvoerbaarheidsondersoek het van kriteria soos doeltreffendheid, implementeringsgemak, finansiële implikasies en omgewingsimplikasies gebruik gemaak, en die vergelyk met die individuele prosesse.

’n Batch eksperimentele opstelling is ontwikkel wat ’n voer behandel het met ’n kombinasie metaalione en ’n oksideringsagent. Die voer het bestaan uit water wat kunsmatig besmet was met bakterie *Pseudomonas sp. strain CT07* teen ’n konsentrasie van tussen $0.5x10^7$ en $2.0x10^7$ cfu/ml. Uitplating is gebruik om ontsmetting te bepaal deur die vermindering van bakteriese konsentrasies tot op ’n vlak onder die opsporingsgrense. Koper-, silwer- en sink-ione is deur ’n ioniseerder vrygelaat en ’n BCDMH voorraadoplossing is as oksideermiddel gebruik. ’n Vaste kontak tydperk van 5-minute is gebruik om die bevindings relevant te hou tot punt-van-gebruik waterdisinfeksie. Eksperimentele resultate was geanaliseer deur gebruik te maak van logistieke regressie.

Die logit-model vir gekombineerde behandeling het ’n sterk korrelasie met ’n Cox-Snell R^2 van 0.516 gehad wat beduidend was met ’n p-waarde <0.001. Die interaksie koëffisiënt (β_{12}) was beduidend met ’n p-waarde van 0.036. Die beduidende interaksie koëffesiënt het getoon dat metaalione die ontsmettingsvermoë van BCDMH, vir ’n kort kontaktydperk van 5-minute, kan verbeter. Die byvoeging van metaalione verminder die hoeveelheid BCDMH wat benodig word om ’n sekere waarskynlikheid te bereik vir suksesvolle ontsmetting volgens die waarskynlikheidsmodel.

Die gekombineerde ontsmettingsproses is meer doeltreffend, kan maklik implementeer word en dit het omgewingsvoordele oor die individuele prosesse. Maar, die gekombineerde tegnologie is duurder om te bedryf as slegs BCDMH-behandeling. Die byvoeging van metaalione deur ionisering kan lei tot ’n 25.67% vermindering in die gebruik van BCDMH, maar dit kom teen ’n bedryfskoste 2.5 keer meer as behandeling sonder metaalione.

Finale ORP waardes het geen korrelasie met die ontsmettingssukses gehad nie. Die verandering in ORP vir die BCDMH behandeling het ’n beduidende verhouding getoon met behandelingsukses met ’n p-waarde van 0.018. Die verhouding het getoon dat ’n ΔORP van 164.35 mV ooreen behoort te stem met ’n 90% waarskynlikheid vir suksesvolle behandeling. Die maksimum eksperimentele ΔORP was egter slegs 117 mV.

Die navorsing het getoon dat metaalione die onsmettingsdoeltreffendheid van BCDMH verbeter. Die tegnologie het verskeie voordele, maar dit is nie finansieel regverdigbaar nie as gevolg van die toename in koste teenoor standaard BCDMH behandeling. Die verandering in ORP was nouer verwant aan ontsmettingsukses as ’n enkele ORP-waarde. ’n Volledige koonsentrasie- kontak tyd (CT) ondersoek sal die begrip van die interaksies tussen metaalione, BCDMH en patogene verbreed.

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Glossary

I. Abbreviations

AA – atomic absorption

ABNC – active but non-culturable

AWWA – American Water Works Association

B.C. – Before Christ

BCDMH – bromo-chloro-dimethyl-hydantoin

CDMH – chloro-dimethyl-hydantoin

cfu – colony forming bacterial units

CT – concentration and contact time

DBP – disinfecting-by-product

DC – direct current

DMH – dimethyl-hydantoin

DNA – deoxyribonucleic acid

DOC – dissolved organic carbon

DSA – Dimensionally Stable Anodes

EC – Electric conductivity

EOW – electrolysed oxidising water

EPA – Environmental Protection Agency

GAC – granular activated carbon

GDWQ – Guidelines for Drinking-Water Quality

HAAS – halo acetic acids

HI – Hanna Instruments

HPC – heterotrophic plate count

IARC	– the international Agency for Research on Cancer
ICP-MS	– inductively coupled plasma mass spectrometry
ISO	– International Organisation for Standardization
LRV	– log removal value
MDGs	– Millennium Development Goals
NASA	– National Aeronautics and Space Administration
NOM	– natural organic matter
OD	– optical density
ORP	– Oxidation Reduction Potential
RO	– reverse osmosis
SABS	– South African Bureau of Standards
SANS	– South African National Standards
SDGs	– Sustainable Development Goals
TDS	– total dissolved solids
THM	– Trihalomethanes
TSB	– Tryptic Soy Broth
TSA	– Tryptic Soy Agar
UN	– United Nations
UNESCO	– United Nations Educational, Scientific and Cultural Organization
USA	– United States of America
UV	– ultra violet
WHO	– World Health Organisation
WWAP	– World Water Assessment Programme
ΔORP	– change in ORP

II. Chemical formulae

Ag	– silver
Ag^+	– silver cation
AgCl	– silver chloride
$AgNO_3$	– silver nitrate
Br	– bromine
Br^-	– bromide or bromine anion
$Ca(OCl)_2$	– calcium hypochlorite
C_2H_6O	– ethanol
$C_3H_8O_3$	– glycerol
$C_5H_6BrClN_2O_2$	– BCDMH
Cl	– chlorine
Cl^-	– chlorine anion
ClO_2	– chlorine dioxide
Cu	– copper
Cu^+	– copper (I) cation
Cu^{2+}	– copper (II) cation
H	– hydrogen
H_2O_2	– hydrogen peroxide
HNO_3	– nitric acid
H_2O	– water
H_2S	– hydrogen sulphide
HOCl	– hypochlorous acid
HOBr	– hypobromous acid
$KMnO_4$	– potassium permanganate

Na – sodium

NaCl – sodium chloride

NaClO – sodium hypochlorite

OBr^- – hypobromite anions

OCl^- – hypochlorite anions

O_3 – ozone

O_2 – oxygen gas

OH – hydroxyl

Pt – platinum

Zn – zinc

Zn^{2+} – zinc (II) cation

$ZnCl_2$ – zinc chloride

III. Pathogen names

A. baumannii – *Acinetobacter baumannii*

C. parvum – *Cryptosporidium parvum*

E. aerogenes – *Enterobacter aerogenes*

E. coli – *Escherichia coli*

E. histolytica – *Entamoeba histolytica*

G. lamblia – *Giardia lamblia*

L. pneumophila – *Legionella pneumophila*

M. avium – *Mycobacterium avium*

P. aeruginosa – *Pseudomonas aeruginosa*

P. cepacian – *Pseudomonas cepacian*

S. bongori – *Salmonella bongori*

S. enterica – *Salmonella enterica*

S. maltophilia – *Stenotrophomonas maltophilia*

IV. Measuring units

°C	– degree Celsius
L	– litre
mL	– millilitre
µL	– microliter
min	– minutes
A	– ampere
mA	– milliampere
V	– Volts
mV	– millivolts
g	– gram
mg	– milligram
mol	– mole
ppm	– parts per million
ppb	– parts per billion
Psi	– pounds per square inch
mS/cm	– milli-Siemens per centimetre
µS/cm	– micro-Siemens per centimetre
$	– Dollar
R	– South African Rand

1.Introduction

1.1 Background

Water is a critical constituent for life to exist. Water does not only play a role in creating a habitat that supports life, but it is one of the building blocks found in all living things (Project WET Foundation 2010). For humans, water has also become essential in the daily agricultural, industrial, and domestic activities that allow society to function (United Nations 2015). With more than two-thirds of the earth covered with water, water does not appear to be a scarcity, but less than 0.5% thereof is available in a form which is fresh, clean, and safe for humans to use (Martin, Fry et al. 2005). Proper and effective management of the available water is therefore a necessity to ensure water demands are met in a sustainable manner.

The human demand for water requires water of a certain quality. Fresh water is one of the major requirements for many water dependent activities, but there are several other water contaminants that often make water unsuitable for its use. These contaminants include heavy metals, organics, nutrients, and pathogens (Bennett 2008). Water quality standards have been developed by governments and international organisations that specify acceptable contaminant levels for specific water uses (EPA 1999). Water intended for human consumption must be strictly assessed and controlled, because pathogens and toxic substances can cause sickness and even death (UNESCO-WWAP 2012). The quality of drinking water available to a community has a direct effect on its population's quality of life.

The fresh water available for human use is quickly deteriorating in quality as current sources are being stressed to their limits due to anthropogenic causes. Population growth has not only led to an increased demand on the limited available water, but has also worsened the state of the water sources due to pollution. Urbanisation, poor sanitation, increased industrial activities, and poor water management are just a few of the direct contributors that have increased the stress on the available water (Scheren, Zanting et al. 2000). Water quality has worsened to the state that about half of the earth's population do not have access to safe and reliable drinking water (UNESCO-WWAP 2012). Few natural water sources can still be used by humans without the risk of exposure to pathogens (Shannon, Bohn et al. 2008).

The quality of water sources has necessitated the need to manage and treat water, specifically the disinfection of water for potable use. The management of water includes all activities that are needed from supplying water to consumers, to releasing used water back into the environment. Water

treatment is done to improve water quality and ensure it is of the required standard before it is used and before it is released back to natural sources. Water disinfection is the inactivation of pathogens in water and forms part of pre- and post-water treatment processes (Denyer, Stewart 1998). Poor and inadequate water disinfection often leads to disease outbreaks, such as cholera, which are caused by waterborne pathogens (Bennett 2008).

Since the discovery of micro-organisms and pathogens in the late 1800's, a variety of water disinfecting technologies and methodologies have been developed (Kim, Anderson et al. 2002). The diversity of disinfecting technologies currently on the market include simplistic processes, such as filtration, to complex processes, such as radical formation. Chlorine based technologies have been the most commonly used disinfectants due to the availability of the chemicals, effectiveness against a wide variety of pathogens, and ease of treatment (Leopold, Freese 2009). Due to the disadvantages of chlorine treatment, such as by-products forming and operational hazards, other disinfecting technologies, such as UV, ozone, and electro-chemical processes, have gained popularity (Kim, Anderson et al. 2002).

With the global deterioration of water, more funding and research are invested in the development of sustainable water disinfection processes that can serve as alternatives to chlorine disinfection (Prüss-Üstün, Corvalán 2006). Characteristics that are being investigated can broadly be grouped as disinfecting efficiency, ease of operation, financial implications, and environmental impact. The disinfecting efficiency refers to, amongst others, the robustness of a disinfectant to deactivate a wide variety of pathogens, how likely it is that resistant pathogens will develop, and to what extent the process has secondary disinfecting capabilities (LeChevallier, Au 2004).

Ease of operation look at characteristics such as how easily the process can be implemented, what skills operators require, and the risks involved for the process implementation. The aim of financial investigations is to decrease capital and operation costs as much as possible without increasing health risks or decreasing water quality. The environmental effect considers the current and future dangers of the treatment method, on the water consumer and the environment. Currently, not a single known disinfectant demonstrates only strengths in all these categories, which has led to the implementation of multi-barrier or combined disinfecting technologies that build on the strengths of the individual processes (Bennett 2008).

A large group of disinfectants are oxidising in nature, i.e. they attack and react with the pathogen structure through an oxidation reaction (Singer, Reckhow 1999). Generally, oxidising agent are more effective as disinfectants than non-oxidising agents (Kim, Anderson et al. 2002). Oxidising agents usually follow an inverse concentration-contact time relationship to achieve disinfection, usually

disinfecting within minutes (Bennett 2008). A large proportion of disinfectants available on the market are oxidising agents, these include chlorine, ozone, chlorine dioxide and hydrogen peroxide. Alternative chemicals are being developed to overcome the challenges posed by chlorine treatment, for example bromo-chloro-dimethyl-hydantoin (BCDMH). BCDMH is a less known organic compound that is more stable than typical oxidising agents; BCDMH slowly reacts with water to release chlorine and bromine species that act as biocides (Moffa, Davis et al. 2006).

Metals have been known to have biocidal properties for centuries, with silver and copper containers used to store water in ancient civilisations (Demling, Desanti 2001). Today, metals, specifically silver, are implemented as biocides in colloidal form, as metal ions, or as nanoparticles (Lin, Vidic et al. 1996). Metal particles inactivate pathogens by attacking their structure, similarly to oxidising agents, but generally require hours or days to ensure successful disinfection. Metal ions can be released through the addition of metal salts or through an electro-chemical process referred to as ionisation (Liau, Read et al. 1997). The ionisation of copper, silver, zinc, copper-silver, and similar metal combinations have been researched as alternatives to chlorine disinfection and can be found on the market (Zheng, Dunets et al. 2012).

The different disinfecting technologies have different advantages and disadvantage, which make them appropriate for different treatment conditions. By combining different technologies, the strengths of the individual processes can be enhanced and the shortcomings mitigated (Meireles, Giaouris et al. 2016). Several disinfecting technologies are currently being implemented together, such as UV with chlorine and ozone with hydrogen peroxide (Shannon, Bohn et al. 2008). The combination of different disinfecting processes can either decrease the efficiency of the individual processes, have a non-interactive additional effect, or can interact to have a much stronger combined effect.

The combination of metal ions and an oxidising agent as disinfectant is discussed in literature, but to a limited extend (Martínez, Gallegos et al. 2004). Metal ions and oxidising agents both attack and weaken cell membranes and walls as part of their disinfecting mechanisms, but through different chemical reactions. Oxidising agents often require extremely short contact times, i.e. minutes, compared to long contact times required by metal ion treatment, i.e. hours. Combined technology do not only widen the range of pathogens that can be deactivated, but could have improved biocidal properties against certain pathogens (Sambhy, MacBride et al. 2006). Combined ionisation-oxidation technology has been used more in industrial applications, than what it has been researched in scientific studies (Fewtrell 2014).

Aquaking SA (Pty.) is a small water treatment company in South Africa that make use of an ionisation-oxidation treatment procedure for disinfection. The technology first releases silver, copper, and zinc

into the water through an electro-chemical cell connected to a copper-silver-zinc electrode. The water then treated with the oxidising agent BCDMH (Aquaking SA 2016). The process is currently effectively implemented in the poultry industry and is often used in fruit packing stores and cooling towers. The technology has had a high success rate as disinfectant - claiming to yield financial and environmental benefits to customers. The ionisation-oxidation process seems to be a valuable component of their technology, although the disinfection interaction is understood to a limited degree (Aquaking SA 2017).

Generally, literature and technology developers both claim that ionisation-oxidation technology is more effective than the individual processes. Silver-copper ionisation combined with chlorine treatment showed higher log reduction than the individual processes added together for point-of-use treatment (Yahya, Landeen et al. 1990). Silver treatment combined with hydrogen peroxide showed synergistic disinfection and a decrease in by-products forming when investigated as a secondary disinfectant method (Pedahzur, Lev et al. 1995). Water treatment companies, especially pool treatment companies, often market ionisation technology as an addition to chlorine treatment with the benefit of a decrease in the chlorine needed (Carefree Clearwater 2015).

The contributions of the separate components of an ionisation-oxidation disinfection system are largely unknown (Yahya, Landeen et al. 1990). Oxidation processes differ to such an extent that improved disinfection, if any, with metal ions will have to be investigated for every oxidising agent. The contribution different metal ions have on a combined process have also not been researched. The optimisation of such a combined technology is of importance in order to decrease costs and any negative environmental impacts. The Aquaking technology that combine silver-copper-zinc ionisation with BCDMH show disinfection, but the contributions of the different components are unknown (Aquaking SA 2017). Understanding of the individual contributions of the components can lead to the optimisation of the disinfecting process.

The need to control and assess disinfection is an important part of water treatment, disinfection, and providing water of acceptable quality. The aim of disinfection assessment would be to ensure there are no pathogens in treated water and neither any disinfecting by-products. Ideally, bacterial concentrations should be measured continuously to ensure complete disinfection, but determining bacterial concentrations through microbiological procedures are time consuming and expensive (Tanchou 2014). In practise, disinfection is rather controlled by monitoring the disinfectant dose or disinfectant residual. In most cases, it is possible to quantify the disinfectant applied, but the

effectiveness thereof is still limited by the levels of contamination, pH, and temperatures (Bastian, Brondum 2009).

When working with oxidising disinfectants, it is difficult to ensure adequate disinfection without spikes in treatment or overdosing due to the inconsistencies in disinfectant demand. Chlorine treatment is usually controlled by measuring the free chlorine residual in combination with the pH. Free chlorine measurements have several drawbacks including being prone to inaccuracy as it is in practice usually measured intermittently by human operators which lead to over- and under-treatment (Devkota, Williams et al. 2000). Automated technology that monitor water quality and triggers disinfectant release immediately when water quality is not within standards, would lead to financial benefits and improve general safety (Ndegwa, Wang et al. 2007). Among others, oxidation reduction potential (ORP) is emerging as a control implemented to monitor the need for oxidation treatment of water.

ORP measures the water's ability to act as an oxidising agent and is affected by the oxidising agents in the water (Sigg 2000). ORP is therefore an indication of the active oxidising disinfectants in the water and can be correlated with its biocidal potential (James, Copeland et al. 2004). Most pathogens are inactivated within seconds in water with ORPs above 650 mV (Suslow 2004). The main advantages of ORP is that it can be used to continuously monitor water quality and activate treatment when necessary. ORP is, however, said to be more a qualitative than quantitative indicator of water quality and is not currently implemented widely as the main disinfectant control (Thomas 2006). There is no literature on using ORP to monitor a dual ionisation-oxidation disinfection process.

1.2 Research problem

The general quality of water requires water to be treated and disinfected before humans can use it. Chlorine is currently the most commonly used water disinfectant, but it has many disadvantages. Alternative water disinfection processes need to be developed that has similar advantages to chlorine treatment, but with fewer drawbacks. Metal ions and oxidising agents have been implemented as combined technology, but the contributions of the individual processes are not well understood. The disinfecting contribution of different oxidising agents and metal ions in combined technology need to be investigated to gain an understanding of ionisation-oxidation disinfection. This can lead to optimisation of ionisation-oxidation processes and the development of alternative disinfectants to chlorine.

There remains a need for the development of continuous disinfection monitoring processes that can ensure optimal treatment and a reliable water quality. ORP is an indication of the oxidising ability of a solution and is used to monitor oxidising disinfection in some cases. ORP is not used extensively for

water treatment for a variety of reasons, such as the complexity of the oxidising agents in water. The potential of ORP to monitor multi-barrier or combined oxidation disinfection processes is unknown.

1.3 Objectives

1) To identify the contribution, if any, of metallic ions on the disinfection ability of BCDMH.
2) To investigate the feasibility of using metallic ions with BCDMH as disinfectant, criteria included:
   a) The disinfection efficiency of the combined technology.
   b) The ease of implementation of the combined technology.
   c) The financial implications of the combined technology.
   d) The environmental implication of the combined technology.

   These criteria were used to compare the combined technology with the individual processes and with general chlorine disinfection.
3) To evaluate ORP as an indicator of disinfection efficacy for a disinfection process that combine metal ions with BCDMH.

1.4 Approach

The first step to reach the objectives was to determine the experimental methodology. A lab scale batch apparatus was designed that was based on the ionisation-oxidation treatment process used by Aquaking SA (Pty.). The batch system was designed to represent a closed volume of contaminated water which would then be treated and tested for successful disinfection. Disinfection success was determined by testing for the presence of bacteria through plating. The water was artificially contaminated with the bacterial pathogen *Pseudomonas sp. strain CT07,* before being treated by BCDMH, or metal ions, or a combination of both. BCDMH treatment involved the addition of dissolved BCDMH solution, while metal ion treatment involved the release of metal ions through an electro-chemical cell. The environmental-related variables temperature, volume, flow and bacterial concentrations were fixed as to represent point-of-use water disinfection.

After determining the experimental setup, the two treatment processes were investigated separately. This involved looking at BCDMH as disinfectant and determining the concentration-time regime where BCDMH is active as disinfectant. Similarly, metal ions were also investigated as disinfectant on their own to determine a treatment-time regime for effective disinfection. The information from the individual treatment processes were used to design the combined treatment experiments that focused on BCDMH as disinfectant and the ability of metal ions to improve disinfection for a point-of-use application.

The experimental results were binary, i.e. either successful or unsuccessful disinfection. The binary data was analysed using logistic regression. A 3D model for the probability for successful treatment was constructed from the logistic regression. The logistic regression data made it possible to determine the contribution of BCDMH concentration and ionisation separately and combined on disinfection. It was also possible to determine any interaction between the different disinfectants from the regression model. The disinfection efficiency and ease of implementation was discussed using literature, industry sources and the experimental data. The logistic regression models were used to optimise the financial cost and limit the environmental impact for such combined technology.

In parallel with the experimentation, ORP was monitored to investigate the potential for a continuous control for water disinfection. Final ORP values, overall change in ORP, and change in ORP for the oxidation treatment, were all compared to final disinfection success. Temperature, bromine residual, pH, and electric conductivity are four other characteristics that were monitored in conjunction with ORP.

1.5 Scope and limitations of research

The focus of this research was to develop an understanding of the disinfection process that combines silver, copper, and zinc ions with BCDMH through the generation and analysis of data according to a proposed experimental design. The data and resulting models were then used to optimise the technology from a financial and environmental objective, and to comment on the feasibility of the technology as an alternative disinfectant to chlorine treatment. Only BCDMH concentration and ionisation time were used as independent variables while all other conditions were kept within bounds to simulate point-of-use disinfection. The microscopic interaction between the metal ions, oxidising agents and pathogens were discussed theoretically and not investigated experimentally.

Metal ion concentrations and BCDMH concentrations were investigated according to treatment concentrations applied industrially and limitations imposed by environmental regulations. The contact times investigated were according to practical point-of-use treatment and more related to oxidising disinfection. Neither BCDMH nor ionisation were fully investigated individually as disinfectants. The separate treatment processes were only investigated to get the necessary information to develop an understanding of the combined treatment process. The combined treatment process investigated did not include different contact times or different contamination levels. Disinfection was simplified to binary results, i.e. either successful or unsuccessful disinfection, and not measured by bacterial log reduction.

The financial investigation was done as an indication of operating costs for the different treatment systems and not a full cost analysis. The financial investigation focused on the operating cost directly related to the chemicals used and would have become overly complex to include infrastructure costs and other possible costs. The environmental impact was also simplified to get a quick indication of the environmental effect without doing a full environmental impact assessment. The feasibility of the technology serves as a short investigation to assess the potential for practical application of the technology as an alternative disinfectant.

The research served to fill a gap in the understanding of ionisation-oxidation disinfection. This will hopefully help future research and help develop the understanding of alternative disinfecting technologies. The research was not supposed to completely explain ionisation-oxidation disinfection.

1.6 Thesis organisation

The second chapter presents the literature reviewed for the research conducted. The chapter starts by looking at water as a resource and the current state of water. The second section of the literature discusses the historical development of water treatment and water disinfection and how society has arrived at the current treatment procedures. Water quality is then discussed, including water contaminants and standards. The core of the literature is focused on water disinfection and different disinfection technologies, focusing on ionisation, oxidation, and ionisation-oxidation treatment. The final section looks at methods of assessing and controlling disinfection, concentrating on emerging ORP technology.

Chapter three presents the experimental methodology. The first section summarises the experimental process followed, before the understanding behind the process is discussed in further sections. The development of the feed conditions for treatment is presented followed by the development of BCDMH treatment and metal ion treatment. The controls and methods of assessing disinfection are discussed before the complete apparatus and experimental procedure is explained. The final section explains the statistical tools that were used to analysis the experimental data.

The fourth chapter is a combination of all the results and discussion of the results. The results of different treatment methods investigated are given. The results are interpreted for the independent BCDMH and metal ion treatment, and for the combined treatment. The results of ORP as a control method to monitor disinfection is also presented and discussed. Chapter five combines all the results and discusses the feasibility of the ionisation-oxidation technology looking at efficiency, ease of implementation, financial optimisation, and environmental implication. Conclusions are drawn in chapter six with mention of the limitations of the research and recommendations for future research.

2.Literature review

The first section, *2.1 Water as a resource*, discusses our dependence of water, its availability, and the need to manage it sustainably. *2.2 Historical development of water treatment* looks at how water management has grown from pure water procurement to full water treatment systems that we have today. The need for water of a certain quality, the contaminants that worsen water quality, and the water standards that have developed are discussed in *2.3 Water quality*. Water disinfection is discussed in *2.4 Water disinfection*, describing a large variety of disinfecting technologies and then explaining oxidising agents and metal ions in detail. The literature builds up to 2*.4.6.2 Metal ions and oxidising agent combinations*, which discusses the application of metal ions with different oxidising agents as disinfecting technology. Different tools for assessing and controlling disinfection are discussed in *2.5 Assessment and control of water disinfection*, with an in-depth explanation of ORP.

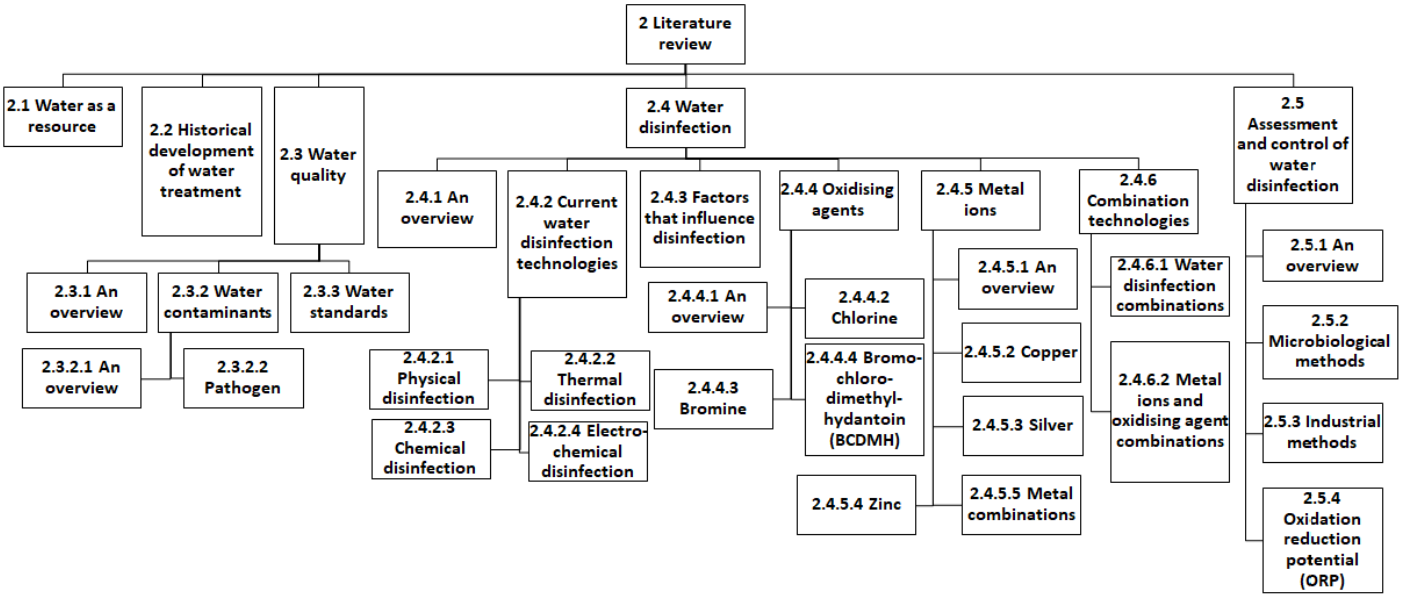

Figure 1: Literature review structure

2.1 Water as a resource

The earth has several special characteristics that make life possible. One of these characteristics is the presence of water. Water, in combination with oxygen and carbon dioxide, forms part of the life support system on earth and without these molecules life will not be able to exist as we know it. All forms of life are dependent on the quality and volume of water available for their survival (Postel, Richter 2012). Aquatic life requires water as habitat, but terrestrial life is as dependant on water, with no living organism being able to function without it. The human body, is an example with about 60%

of it comprising of water, therefore, water is critical in the functioning of the human body (Project WET Foundation 2010).

Water is not only necessary for its direct contribution to the life cycle, but it forms part of most daily life activities. Most domestic, industrial, and agricultural activities require water to function normally (United Nations 2015, Molden 2007). Domestic water uses include drinking water, washing, cooking, general sanitation, and recreational activities (Hall, Van Koppen et al. 2014). Industrial activities often require water to form part chemical processes, cleaning, and cooling procedures. Agriculture and food production is the most dependent on water and the largest consumer (Molden 2007, United Nations 2015). According to the 2015 Millennium Development Goals Report, municipalities account for 12% of the freshwater withdrawal, industries account for 19% and agriculture, mainly irrigation, account for 69% of fresh water withdrawal (United Nations 2015).

Although water is abundant on Earth, 71% of the Earth's surface is covered in water, the water that is usable is limited (Factbook 2010). Only 3% of the water on Earth is fresh, and 2.5% of the fresh water is frozen in the Arctic and Antarctic which leaves only 0.5% available for humans to use (Martin, Fry et al. 2005). The 0.5% fresh water available is divided unevenly around the Earth as ground and surface water. Figure 2 shows the proportional divide of fresh water sources compared to all water on Earth. Most human activities and most terrestrial life, including some aquatic life, is dependent on this small percentage fresh water.

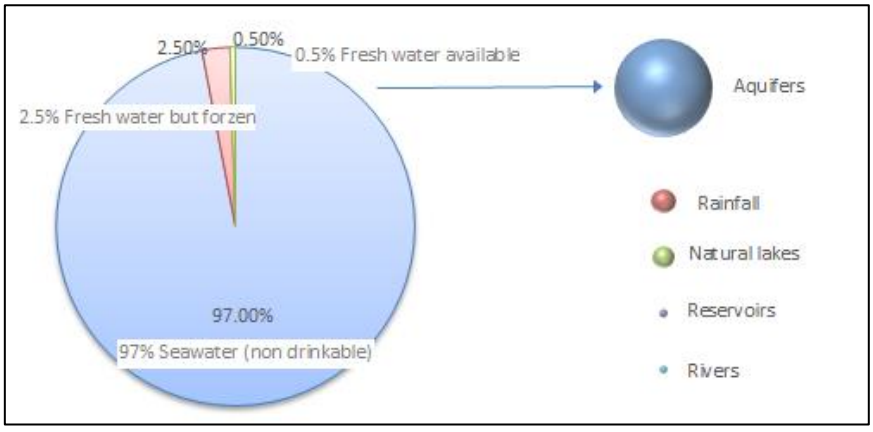

Figure 2: Proportional divide of available fresh water redrawn from Martin, Fry et al. (2005)

The available water is not only limited but is being stressed as resource. It has been estimated in 2015, that more than 40% of humans on the globe are affected by water scarcity (United Nations 2015). This percentage will increase as water pollution increases, natural disasters such as drought and flooding hit populated areas, and general poor management of available water and waste water continue (Postel, Richter 2012). Poor farming techniques, mining and industrial activities, and domestic

pollutants are the main contributors to surface- and groundwater pollution (Scheren, Zanting et al. 2000). Environmental regulations have been set in place to enforce proper management of liquid, gaseous and solid waste to try limit the contamination of the fresh water available, but higher water demands are continuously adding stress to water supplies (Martin, Fry et al. 2005).

There have been several initiatives to improve water quality and decrease the stress on water. The biggest challenge is to meet the growing water demand with an already limited water source. The Millennium Development Goals (MDGs) initiative, which has now been replaced by the Sustainable Development Goals (SDGs), has insured improvement in water provision across the globe, with 91% of the global population having access to improved drinking water sources according to the 2015 report (United Nations 2015, United Nations 2017). However, in many of these cases the quality of the water still does not comply with the required standards, with more than 80% of water used globally not being treated before being used (Corcoran, Nellemann et al. 2010). When comparing the supplied water to modern water standards, a 2012 study estimated that 3-4 billion people, about half the Earth's population, did not have access to safe and reliable drinking water (UNESCO-WWAP 2012).

According to a 2008 study, it was estimated that every year 3.5 million people die due to inadequate water supply, poor sanitation, and poor hygiene (WHO 2008). Human health has deteriorated rapidly in developing countries as pollution has worsened, but water treatment and supply has not been developed as rapidly (Molden 2007). Poor areas in developing countries often have water sources that does not even seem palatable, but is used as a potable water source. It is estimated that 10% of all disease cases could be cut by improving water supply, sanitation, and hygiene (UNESCO-WWAP 2012). Inadequate water supplies do not only effect health, but effects food quality, social structures, and the economic productivity of communities (Gleick 1998, Postel, Richter 2012, Hall, Van Koppen et al. 2014).

Water treatment has become a non-negotiable issue. The United Nations General Assembly made the point official when they decided in 2010 that clean and safe drinking water and sanitation is a basic human need (Hall, Van Koppen et al. 2014). Financially, it makes logical sense to improve water quality, as the global financial return for investing in water supply and sanitation is estimated at $8 to every $1 invested (Prüss-Üstün, Corvalán 2006). Improved drinking water, sanitation systems and hygiene benefit everything from personal health to communities' economies. The management, and specifically the treatment, of water is critical in ensuring water for the generations to come.

2.2 Historical development of water treatment

The first records of water purification dates to the 20th century B.C., Sanskrit writings make mention of copper vessels, exposure to sun, and filtration through charcoal as methods to treat water. At around the same time in history, it seems that the Chinese made use of heat as a water purification method by boiling water. Throughout the ancient times, the focus was much more on water procurement and storage, than on water purification and treatment. In the 5th century B.C. Hippocrates observed that "… water contributes much to health …" and he recommended that "… rain water be boiled and strained though a cloth bag otherwise they could have a bad smell …" (Symons 2006). Only 23 centuries after the first records of water treatment did water treatment and disinfection become the norm.

The understanding of the importance of water purification developed with the growing knowledge of pathogens. Before the 19th century palatable water, i.e. water that looks drinkable, smells good and tastes good, was used as drinking water (Symons 2006). Only when Louis Pasteur started to get support for his research on micro-organisms, did society start to realise the difference between potable and palatable water. In 1854, before Pasteur's first publication, John Snow was the first to investigate and proof that a community's drinking water was the source of a spreading disease (AWWA 1971). Pasteur's publications in the second part of the 19th century finally convinced scientists, society, and health practitioners, that micro-organisms existed and that some of these micro-organisms cause diseases (Debre 1998).

With the industrial revolution and urbanisation in the 18th and 19th centuries, the quality of surface water quickly deteriorated. By the start of the 19th century public works- and health administrators recognised the need for filtration as rivers, dams and other water sources became unpalatable. The first sand filters implemented were in 1829 in London (AWWA 1940). By the end of the 19th century it was understood that bacterial content in water influenced the quality of the water, but only by 1906 did the concept of a disinfectant to destroy pathogens find credibility (Turneaure, Russell 1906). From there disinfection technology started developing rapidly. A report written in 1927 mentioned the use of ozone, UV rays and iodine, but praised chlorine as the "…most widely used disinfectant" (Flinn, Bogert et al. 1927).

The standardisation of water quality in 1914 in the USA spurred the development of water treatment technology, and specifically water disinfectants (McGuire 2006). When water providers were required to comply with water standards, it made economically sense to start researching different disinfectants and their effectiveness on bacterial inactivation. The standards enforced focused on

contaminants that were known, which were mainly bacterial pathogens, and were measured by the presence of coliform bacteria (Symons 2006). Water standardisation developed continuously as a wider variety of water contaminants and pathogens were identified (McGuire 2006). Today, different water uses require different water standards which are implemented by local authorities and international organisations.

As research technologies improved and developed, other forms of water contaminants, and specifically pathogens, were identified. Water purification in the beginning of the 20th century focused on purifying water from bacterial pathogens because the common waterborne diseases, such as cholera, traveller's diarrhoea, and typhoid fever, were all caused by bacterial cultures (Symons 2006). In 1945 viruses were identified as another possible cause for waterborne diseases and the understanding of viruses developed until 1970, when it was proven that the then used disinfectants were effective against viruses. The identification of the parasitic protozoa *Giardia lamblia*, in the 1970's, made scientists aware of water contaminants that could be present in water that was believed to be free from pathogens. Generally parasitic protozoa showed greater resistance to the disinfectants used at that time, which stimulated research into alternative disinfectants (Trussell 2006). The outbreak of cryptosporidiosis in 1993, caused by a highly resistant protozoon named *Cryptosporidium*, highlighted the limitations of chlorine water treatment and the continual need for research (McGuire 2006).

In 1974 a different danger of chlorine disinfection was identified. The formation of trihalomethanes (THMs) were discovered with the chlorination of water containing natural organic matter (NOM) (McGuire 2006). THMs were identified as poisonous to humans and the use of free chlorine as disinfectant was decreased dramatically with immediate regulations regarding the concentration of free chlorine which could be in water (Trussell 2006). It is now believed that THMs are carcinogenic (Duke, Siria et al. 1980), influence fertility and have a direct effect on foetal development in pregnant mothers (Wright, Schwartz et al. 2003). As research continued, other disinfection by-products (DBPs), such as halo acetic acids (HAAs), have also been identified (Hua, Yeats 2010). The Environmental Protection Agency (EPA) has established concentration limits for DBPs which has led to the research of alternative disinfectants such as ozone, UV, and other oxidising disinfectants (EPA 1999b).

To ensure water quality and compliance with regulations, water quality indicators were put in place (McGuire 2006). For the first half of the 20th century, total coliform and turbidity were mainly used as indicators for successful treatment. These indicators were simplistic and easily implemented, but were not a true indication of potable water (McGuire 2006). The identification of other contaminants and pathogens has led to specific treatment protocols for every contaminant to ensure adequate

treatment. Chlorine treatment, for example, is monitored by measuring free-chlorine residual and pH. Every component of a water treatment process has its own treatment requirements and monitoring process that needs to be implemented to ensure treated water is of the required quality (Meireles, Giaouris et al. 2016, Singer, Reckhow 1999).

Water treatment has developed into a complex process with a variety of components. The basic components of a modern treatment process are summarised in Figure 3. Water is recovered from natural sources and usually screened to remove larger particles. The screening is followed by coagulation and flocculation which causes particles to settle out in the settling tanks. Filtration is then used to remove most contaminants. Water disinfection is finally applied to remove any pathogens and to prevent recontamination. Depending on the water quality of the influent and specific water contaminants, various other water treatment components can also be added such as a bioreactor, a water softener, and membranes, to mention a few (Cheremisinoff 2001, Schutte, Focke 2006, EPA 2009, Aaberg Claim Professionals 2012, CDC 2017).

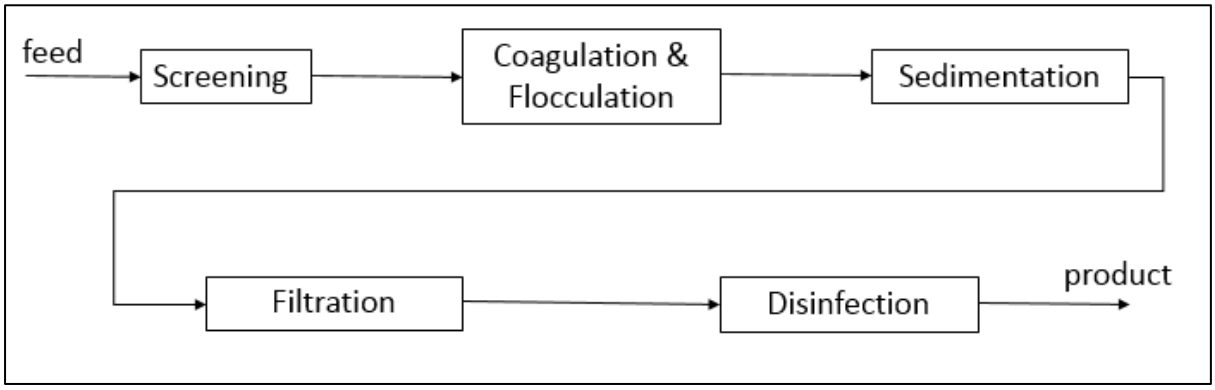

Figure 3: Basic water treatment process

For the largest part of the 20th century chlorine was the most widely used disinfectant (Kerwick, Reddy et al. 2005). This is due to its availability, its efficiency against the known pathogens, and its simplicity to implement in combination with a filter to provide drinking water. The identification of more resistant pathogens, such as *Cryptosporidium* and *G. Lamblia*, and the awareness of by-products formed from chlorine disinfection, has led to the research of numerous other disinfecting chemicals and mechanisms (Trussell 2006, Meireles, Giaouris et al. 2016). Many of these disinfectants have been known about from the beginning of the 20th century, but few had been researched extensively (McGuire 2006). Thermal disinfection, numerous oxidisers and some non-oxidising procedures, as well as combinations of different disinfectants, have shown potential to become alternative disinfectants to chlorine (Coulliette, Peterson et al. 2009).

2.3 Water quality

2.3.1 An overview

Water quality refers to the combination of chemical, physical, and microbiological characteristics of water (Schutte, Focke 2006). Depending on the combination of these different properties, water can either be adequate or inadequate for a certain use. Water for agricultural use, for example, requires a specific pH, hardness, alkalinity, and salinity in which the specific plant species will thrive in (Ayers 1985, Molden 2007). Water used in industrial applications must often be extremely pure to prevent damage to equipment and to prevent the formation of other chemical by-products. Domestic water applications require water that is chemically stable and aesthetic, for general cleaning purposes, and it must be safe for human consumption that can be used as drinking water (Schutte, Focke 2006).

Water contaminants are defined as the things that cause water to decrease in quality. The contaminants are therefore always relative to the water characteristic required by the water demand. These contaminants can be naturally occurring or human caused. Natural contaminants include salts, metals, organisms, and colloidal particles (Schutte, Focke 2006). These contaminants are naturally found in some water sources, but can be increased by poor water management. Human caused contaminants are defined as contaminants that are present because of human activities, such as sewage effluent, industrial waste, and pesticides released into water bodies. Human caused contaminants can be summarised as pollutants which disturb the natural ecosystems and habitats (Cheremisinoff 2001, Fewtrell, Bartram 2001, John, Trollip 2009).

Water standards are put in place to ensure the water quality is improved to meet the requirements of the water application (SABS 2015a). Water users are often ignorant about the effect of water contaminants and therefore larger organisations and governing bodies make it their responsibility to ensure water is of the required quality (John, Trollip 2009, Fewtrell, Bartram 2001). The standards are researched and developed to protect the water users from being exploited by water utilities providing water of inadequate quality. Water standards are usually developed with a focus on protecting human health and ensuring the quality of life. The World Health Organisation (WHO) has three main groups of standards, namely drinking water, wastewater reuse, and recreational water (Fewtrell, Bartram 2001).

2.3.2 Drinking water contaminants

2.3.2.1 Drinking water contaminants: an overview

Water is usually either safe or unsafe for human consumption. As mentioned earlier, it is primarily water contaminants that make water safe or unsafe. However, the lack of minerals in water, i.e. demineralised water, is also believed to be unsafe for humans when consumed over long periods of time (Kozisek 2005). Drinking water contaminants usually either effect the aesthetic value or the health quality of water. Water that has no turbidity and tastes, smells, and looks good is termed palatable. While water that will not cause any health problems, is termed potable (Sigworth 1957). Drinking water standards aim at ensuring humans get palatable and potable water that looks good to drink and which is safe (Fewtrell, Bartram 2001). Several common water contaminants will be discussed briefly in this section. Some of these contaminants are caused by human activities while others are naturally occurring and only worsened by human activities (Schutte, Focke 2006).

Natural water sources typically contain suspended and dissolved contaminants such as clay particles, salts, heavy metals, nutrients, and organic matter. The clay particles usually have no direct health danger for humans, but increase turbidity which makes water unpalatable (Schutte, Focke 2006). Clay particles also complicate water treatment as it may contain heavy metals and often protect pathogens against disinfection processes (Cheremisinoff 2001). Filtration is generally used to remove clay particles. Inorganic salts, such as sodium chloride (NaCl), can be present in high concentrations depending on the minerals found in the rock layers around the water source. Reverse osmosis is generally used to remove salts from water (Schutte, Focke 2006). Heavy metals can be present due to pollution from industrial activities or mining, or can be present in natural water bodies exposed to high metal containing rock layers. Oxidising agents can oxidise small concentrations of heavy metals out of the water (Cheremisinoff 2001, EPA 1999b).

Phosphates and nitrates are both nutrients essential for life, but in high concentrations in water cause eutrophication. Phosphates are a common and essential component of a wide variety of products, from fertilizers to laundry soaps that end up in effluent water (Leopold, Freese 2009). Phosphates increase chorine demand in chlorination treatment and cause electrode fouling in electrolytic systems (Kerwick, Reddy et al. 2005). Nitrates are released into surface and ground water primarily through fertilisers, human waste and animal waste. Nitrates are dangerous to infants and pregnant woman in drinking water. The removal of nitrates is most efficiently done though reverse osmosis, ion exchange units or nitrifying bacteria. The presence of nitrates limits the efficiency of chlorine species (Shannon, Bohn et al. 2008).

Organics refer to carbon containing compounds and are commonly found as organic matter in water. Human activities can also release organic substances such as gasoline, pesticides, and herbicides into water sources (Cheremisinoff 2001). Organics can be oxidised chemically or by bacteria. Organics are measured by the chemical oxygen demand (COD), which refers to the amount of oxygen required to oxidise the organics. Organics therefore limit oxidising disinfecting agents, since the oxidising agents react with the organics more readily than with the pathogens (Meireles, Giaouris et al. 2016). Chlorine species reacting with excess organics can form trihalomethanes (THMs) which is believed to be carcinogenic, can cause infertility and effect foetal development (WHO 2003b, Leopold, Freese 2009).

Microbiological contaminants are organisms, many of them micro-organisms, which cause some negative effect on water users or the environment. *Shannon, Bohn et al.* divided microbiological contaminants into the following groups: helminths, protozoa, fungi, bacteria, rickettsia, viruses and prions (Shannon, Bohn et al. 2008). Not all microbiological contaminants are pathogenic to humans, but could cause other problems related to the water supply, such as bacteria causing corrosion in pipe networks. The more common pathogenic contaminants are bacteria, usually defined as gram-positive or gram-negative, viruses, and protozoan cysts (WIEGEL 1981, EPA 1999a, Cheremisinoff 2001). From the first identification of water pathogens at the end of the 19th century, there has been a continuous emergence of new pathogens (Shannon, Bohn et al. 2008). Microbiological contaminants that have been identified as common water pathogens include *E. coli*, *G. lamblia, salmonella* and *legionella* (Lin, Vidic et al. 1996, Haas, Joffe et al. 1996, Walker, Rogers et al. 1994). Disinfection is the primary method of removal of pathogens (EPA 1999a).

Biofilms are a combination of sessile biotic communities and a variety of abiotic substances that form on wet surfaces. Micro-organisms attach to surfaces in wet environments and form communities while producing extracellular polymeric substances (EPS). The EPS form a favourable growth environment, which protects the biotic community, keep the community together and store nutrients and minerals for cell growth (Kumar, Anand 1998, Walker, Rogers et al. 1994). Bacteria, and other pathogens, embedded in biofilm, develop special phenotypes that can be up to 100 times more resistant to disinfectants than their planktonic counterparts (Kim, Anderson et al. 2002, Delaedt, Daneels et al. 2008). Biofilms cause several problems in pipeline networks which have financial implications, including a decrease in flow rates, drop in water pressure, heat energy loss, and other phenomena such as bacterial induced corrosion (Martínez, Gallegos et al. 2004, Walker, Rogers et al. 1994).

2.3.2.2 Pathogens

Pathogens are disease-causing organisms that are found all around us in the air, on surfaces and in water. Water pathogens are the group of pathogens that are found in water and to which humans get

exposed through water (Schutte, Focke 2006). Pathogens usually have a parasitic relationship with humans, using humans as habitat at the expense of human health. The removal or inactivation of pathogens in water treatment is of the utmost importance to ensure the safety of water users (World Health Organization 2008). This section will look at common water pathogens, what their effect is on humans, the sources of pathogens, how they spread, and how they are detected and removed from water sources.

Most water pathogens form part of bacteria, viruses, protozoa, or helminths (Cheremisinoff 2001, World Health Organization 2008). Bacteria are single-celled microorganisms that are living, but without a nucleus (World Health Organization 2008). Common aquatic bacteria families include *pseudomonas* and *salmonella* (World Health Organization 2008). Viruses are extremely small parasitic organisms that consist of a nucleic acid molecule in a protein coating. Most viruses dangerous to humans can only multiply in humans (Cheremisinoff 2001, World Health Organization 2008). Protozoa are single-celled organisms which include flagellates, amoebas, and ciliates (Alcamo, Warner 2009, Ruggiero, Gordon et al. 2015). Helminths are commonly referred to as parasitic worms, of which the fluke and tapeworm are well-known (World Health Organization 2008). Table 1 is the complete list of bacteria, viruses, protozoa, and helminths that the WHO reviewed as pathogens in the Guidelines for Drinking-Water Quality (GDWQ).

Table 1: Pathogens reviewed by WHO (World Health Organization 2008)

Bacteria	**Viruses**	**Protozoa and Helminths**
Acinetobacter	*Adenoviruses*	*Acanthamoeba*
Aeromonads	*Astroviruses*	*Balantidium coli*
Bacillus	*Caliciviruses*	*Cryptosporidium*
Burkholderia pseudomallei	*Enteroviruses*	*Cyclospora cayatenensis*
Campylobacter	*Hepatitis A virus*	*Dracunculus medinensis*
Cyanobacterial toxins	*Hepatitis E virus*	*Endamoeba histolytica*
Escherichia coli	*Rotaviruses and orthoreoviruses*	*Fasciola spp.*
Helicobacter pylori		*Giardia intestinalis*
Klebsiella		*Isospora belli*
Legionella		*Microsporidia*
Mycobacterium		*Naegleria + Acanthamoeba*
Pseudomonas. Aeruginosa		*Toxoplasma gondii*
Salmonella		
Shigella		
Staphylococcus aureus		
Tsukamurella		
Vibrio		
Yersinia		

Human ingestion of pathogens usually leads to infection of acute diseases with symptoms such as diarrhoea, fever, stomach cramps, and nausea (Schutte, Focke 2006). Individuals with poor immune systems usually struggle to recover from waterborne diseases which can lead to death, if untreated. Diseases related to bacteria, viruses, protozoa, and helminths differ significantly, but when they are waterborne they often infect the gastrointestinal tract (World Health Organization 2008). Bacterial water contaminants usually release toxins that damage the body, examples include typhoid fever, tuberculosis, and cholera (Cheremisinoff 2001, Schutte, Focke 2006, World Health Organization 2008). Generally, viruses cause the destruction of the host cell, examples of viral diseases are hepatitis and rotavirus diarrhoea (Schutte, Focke 2006). Protozoa and helminths are the cause of most animal and human diseases. Many “emerging diseases” are caused by protozoa, including cryptosporidiosis and giardiasis (World Health Organization 2008, Schutte, Focke 2006). Helminths refer to all worms, but many people, especially in developing countries, are infected by roundworms and flatworms (World Health Organization 2008).

Pathogens can be found in surface water and ground water, but is usually present because of human or animal activities. Generally, water pathogens are excreted in animal and human faeces and spread when these faeces contaminate water sources (World Health Organization 2008). The pathogens often do not grow or multiply when in the water source, but use the water source as a carrier to reach a following host. Humans are then exposed to the pathogens through oral intake, skin contact or open wounds (Cheremisinoff 2001). Poor sanitation, wastewater management, and farming techniques therefore lead to increased faecal contamination in fresh water sources. In developing countries, a single waterborne disease case, such as cholera, often leads to large disease outbreaks in the whole community (World Health Organization 2008, Schutte, Focke 2006).

Pathogens are not always easily detected and often require expensive procedures to detect. Indicator organisms have been identified that give an indication of the presence of other pathogens. Therefore, instead of testing for individual pathogens, of which there are thousands, a single test indicates whether other pathogens should be present. Raw water quality and treated water quality can then quickly be determined. Several characteristics, of an ideal pathogen indicator, which have been identified are (Fewtrell, Bartram 2001, Schutte, Focke 2006):

- It should not be present in uncontaminated water, and always be present when the pathogen is present.
- It should be present in animal and human faeces.
- It should respond to changes in environmental and treatment conditions similar to the actual pathogens.

- It should be inexpensive and simple to detect.
- It should be safe to work.

The common pathogen indicators are total coliforms, faecal coliforms, *E. coli*, heterotrophic bacteria, and coliphages (Schutte, Focke 2006). Coliforms are often used to get a general idea of water quality and effectiveness of disinfection because most bacteria pathogens react similarly to disinfectants. Faecal coliforms and *E. coli* are both a subset of coliforms, but give an indication of faecal contamination (Fewtrell, Bartram 2001). Heterotrophic bacteria plate counts give a general indication of microbe activity and treatment effectiveness. Coliphages are viral contaminants that are easier detectable than human viruses (Schutte, Focke 2006). Viruses, protozoa, and helminths are usually not investigated individually because they are too difficult and expensive to detect (Fewtrell, Bartram 2001).

Pathogens need to be removed from water sources through disinfection. Disinfection can take on a variety of forms from physical removal, such as filtration, to chemical inactivation. Pathogen removal or inactivation is usually measured in log reduction, which refers to the number pathogens removed on a logarithmic scale. Log reduction means that the bacterial concentration is decreased by 10 to the power of the log reduction. In other words, 1-log reduction means the bacterial concentration is 10 times smaller than it was, and a 5-log reduction means that the bacterial concentration is 100 000 times smaller than it was. A 2-log reduction would be equivalent to a 99% decrease in bacteria concentration while a 5 -log reduction would be equivalent to a 99.999% decrease in bacteria concentration.

2.3.3 Water standards

To ensure water quality, local and global governing bodies have identified the need to put water quality regulations in place (Fewtrell, Bartram 2001). There are three perspectives from which water quality regulations are put in place, although they are directly influenced by each other and overlap. The first perspective is that of ensuring the health of the end-users, i.e. how to protect and ensure human health. The second perspective is the environmental impact, to minimise any negative effects on the environment. Thirdly there is the management of water as a resource, ensuring water supply meets demand and sustainable management thereof (World Health Organization 2008).

The World Health Organisation (WHO) is a specialised agency of the United Nations (UN) focused on international public health. One of the primary goals of the WHO is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water” (WHO 2003a). The WHO tries to achieve this goal by proposing regulations and to make recommendations with regards to international water quality

(WHO 2003a). The WHO has published a variety of reports on drinking water guidelines, contaminants, and water treatment which are continuously being reviewed and updated to include the latest technology. There are reports discussing chlorination and alternatives to chlorine, which investigate household disinfectants and their performance. These investigations look at reduction of bacteria, viruses, and protozoa as target contaminants measured against expected contamination levels in an area (Fewtrell, Bartram 2001, World Health Organization 2008, WHO 2016).

The Environmental Protection Agency (EPA) is an agency of the federal government of the United States that has been given the responsibility of proposing legislation and recommendations that protects human health and the environment. Water management form a crucial part of the responsibility of the EPA under the Office of Water. The EPA has drawn up numerous guidance documents to help authorities to legislate in a manner that protect the consumer and environment from water resource exploitation. The EPA makes use of a “Maximum contaminant level goal” (MCLG) and a “Maximum residual disinfectant level goal” (MRDLG) which are non-enforceable recommendations. The “Maximum contaminant level” (MCL), “Maximum residual disinfectant level” (MRDL), and “Treatment technique” (TT) are enforceable standards set out by the EPA (EPA 2009).Industries can use EPA manuals to develop their products and processes accordingly (EPA 1999b).

The South African government, provincial government, and city councils all play a role in water management in South Africa. The Water Service Act is the overruling legislation in South Africa regarding water management, and needs to be implemented by the different government bodies. For drinking water, the South African National Standards (SANS) have two documents related to drinking water quality that have last been updated in 2015 (SABS 2015a, SABS 2015b). SANS 241-1 describes the microbiological, physical, aesthetic, and chemical determinants for drinking water and SANS 241-2 discusses the application of SANS 241-1 (SABS 2011a, SABS 2015a). SANS are usually in line with the standards prescribed by the International Organisation for Standardization (ISO). Table 2 compares the guidelines stipulated by the WHO, with EPA standards and SANS 241-1.

Table 2: Drinking water standards for different pathogens and indicators (World Health Organization 2008, EPA 2009, SABS 2011a, SABS 2015a)

Pathogen indicator	WHO guideline	EPA standard	SANS 241-1
Total coliform	No specific values*	<5% of samples must test positive	≤ 10 cfu/100 mL
E. coli or faecal coliforms	Must not be detectable in any 100-mL sample	Compare to total coliform and repeat if present	Not detected
Heterotrophic plate count (HPC)	No specific values*	≤ 500 cfu/mL	≤ 1 000 cfu/mL
Somatic coliphages	No specific values*	No regulation**	Not detected
Giardia lamblia	No specific values*	3-log removal	Not detected
Cryptosporidium	No specific values*	2-log removal	Not detected
Viruses	No specific values*	3-log removal	Not detected

*The WHO GDWQ discusses these pathogens and indicators but give no guideline values (World Health Organization 2008)

**The EPA standard makes no mention of somatic coliphages as a water quality control (EPA 2009)

2.4 Water disinfection

2.4.1 An overview

A large proportion of the Earth's water sources are contaminated and require water to be purified to make it safe for human consumption (Shannon, Bohn et al. 2008). Water purification vocabulary is diverse, therefore unambiguous definitions are required for the used words.

- Water purification refers to the removal of all contaminants, biotic and abiotic, restoring water to its pure form of H_2O.
- Sterilisation is the destruction or inactivation of all biotic organisms, pathogenic and non-pathogenic (Sletten 1974, Dvorak 2005).
- Water disinfection is the term used to describe the removal or inactivation of all pathogenic organisms in water (Leopold, Freese 2009). Disinfectants function as chemical biocides that exhibit poor selective toxicity which can also come across as poor target specificity (Denyer, Stewart 1998).
- Water treatment refers to any process or action applied to water with the intention of making it less hazardous.

The concept of disinfection involves the interaction of a disinfecting agent and some sort of contaminant. When disinfectants and disinfecting agents are considered, numerous things need to be considered. Broadly categorising, disinfectants can be grouped into physical, thermal, and chemical disinfectants (Kim, Anderson et al. 2002, Meireles, Giaouris et al. 2016). Within these disinfecting groups, numerous subdivisions exist that consider the form of the disinfectant, the disinfection

mechanisms, and cellular interactions. Different disinfecting agents react differently with different contaminants, and many times the disinfecting technology consists of numerous disinfecting agents that work in combination. The interaction between the disinfectants and contaminants are controlled by the characteristic of the agent, the contaminant cell morphology, and the physical characteristics of the contaminant (Denyer, Stewart 1998).

The main factors that affect the efficiency of a specific disinfectant is the disinfectant concentration (when relevant), contact time, temperature, and pH (LeChevallier, Au 2004b). These factors are mostly relevant to chemical disinfectants. The ability of a disinfectant to form a residual in the water is important for the treatment of any water that needs to be transported in a distribution network or that will be stored (Leopold, Freese 2009). The disinfectant residual enables secondary disinfection, which refers to sustaining the water quality in the distribution network by preventing recontamination (LeChevallier, Au 2004a). With different water sources used for different applications, the type and seriousness of contaminants differ generally. A more modern approach has been the combination of different disinfecting agents that theoretically fill each other's weaknesses and prove synergetic in operation (Shannon, Bohn et al. 2008). Investigations have shown that the disinfecting mechanisms can be different from the original and expected mechanisms (Williams, Elder et al. 1988). At this point, chlorine remains the most widely used disinfectant (Leopold, Freese 2009, Meireles, Giaouris et al. 2016).

2.4.2 Current water disinfection technologies

2.4.2.1 Physical disinfection

Physical disinfectants make use of a physical mechanism or separation concept. The complexity of technology and mode of action makes it difficult to clearly differentiate between physical and chemical disinfecting agents. For example, some papers classify ultra violet (UV) irradiation as a chemical disinfectant and others as a physical. This section will look at evaporation, filters, membranes, reverse-osmosis (RO), ultra-violet (UV) irradiation and ultrasound (Kraft 2008). These short descriptions serve to give a broad understanding of these disinfection processes.

Distillation is the evaporation and condensation of water and is one of the oldest physical water purifying methods (Symons 2006). It is the natural purification method the Earth uses continuously in the water cycle. The concept of evaporation and distillation is energy extensive and requires specific operating conditions and expensive machinery to operate efficiently (Donaldson 1960, Cheremisinoff 2001). Distillation has historically been used for desalination (Schutte, Focke 2006). The concept is often more used in desperate situations, where individuals purify salt water for survival on a small scale.

Sand filters are well known physical separators used worldwide, but with a limited disinfection efficiency. Filtration is a natural phenomenon which is in action as surface water filter through soil and porous rock layers to form aquifers. Most sand filters do not stop micro-organisms, and therefore the effluent is primarily palatable and not necessarily potable. Membrane filters remove extremely small particles out of water and is therefore more effective in removing pathogens than normal filters (Meireles, Giaouris et al. 2016). Membranes have become more popular in water treatment over the two decades as membrane technology improved (LeChevallier, Au 2004a). Reverse osmosis (RO) is currently the most efficient selective membrane applied in water treatment, but it removes not only toxic contaminates from water, but also the healthy components (Kraft 2008).

Figure 4 compares the approximate sizes of different pathogens with physical removal techniques that are well known. Filter media will only be effective in removing algae, most protozoa, and a large portion of bacteria, but not any viruses. Microfiltration (MF) can remove all algae, and protozoa, most bacteria, but not viruses. Ultrafiltration (UF) can remove all pathogens except viruses. Only Nano-filtration (NF) and reverse osmosis (RO) can remove all bacteria, viruses, protozoa, and algae with certainty (Bennett 2008).

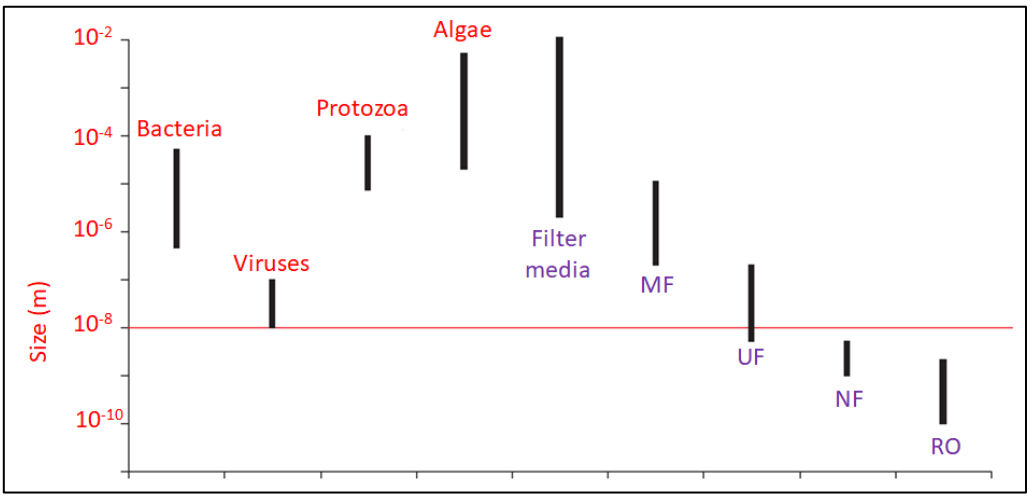

Figure 4: Approximate pathogen size ranges compared to approximate exclusion ranges of physical disinfectants redrawn from Bennett (2008)

Ultra violet (UV) irradiation makes use of light rays with a very high frequency that carries high intensity energy and destroys cells (Lui, Roser et al. 2016). Different studies have proven that different wave lengths can cause the destruction of different micro-organisms. It seems the germicidal efficiency of UV irradiation is dependent on the wavelength which attacks the DNA in the cells (Song, Mohseni et al. 2016, Meireles, Giaouris et al. 2016). Ultrasound makes use of sound waves with frequency above the human-hearing threshold that form cavitation bubbles. These bubbles collapse which release energy that cause the destruction of cells and, therefore, disinfects (Meireles, Giaouris

et al. 2016). Other forms of ionizing radiation, such as X-rays, gamma-rays, and electron beams, result in the formation of radicals that cause disinfection (Meireles, Giaouris et al. 2016). UV disinfection is implemented in water treatment where no residual is wanted, such as waste water released back into the environment (Kim, Anderson et al. 2002).

Most physical disinfection methods require a large physical footprint and are energy intensive (Meireles, Giaouris et al. 2016). While distillation has become financially non-viable, RO technology has similar results but is more implementable, especially when considering wastewater reuse and desalination (Shannon, Bohn et al. 2008). The largest setback to physical disinfectants is the lack of a disinfectant residual, i.e. the processes is only effective as a primary disinfectant and has no secondary disinfecting ability (Kraft 2008, Kim, Anderson et al. 2002). Health experts are also questioning the health implications of drinking water that has no nutrients, salts or minerals (Kozisek 2005). UV irradiation and ultra sound are more financially viable, but are very target specific in application and therefore any physical particles in water can cause a disinfecting shadow where no disinfection takes place. UV has been limited in its efficiency to treat viral pathogens (Shannon, Bohn et al. 2008).

2.4.2.2 Thermal disinfection

Living organisms require certain conditions to live in, and one of these is temperature. At very low and very high temperatures many organisms are destroyed or die, while most organisms are adapted to thrive and grow in temperatures between 20°C and 50°C (Meireles, Giaouris et al. 2016). By increasing water temperatures above 60°C, micro-organisms start to die, until even the most resistant micro-organisms are destroyed (Kim, Anderson et al. 2002). Thermal disinfection focuses on increasing water temperatures to the required levels and maintaining it there for sufficient time to kill the pathogens. Systematic testing has proven at which temperatures water needs to be kept and for what time to kill the different pathogens (Kusnetsov, Iivanainen et al. 2001).

Thermal disinfection has basically no residual as energy is lost to the environment. Therefore, thermal disinfection is limited to treat water that will be used immediately. Water that will only be used some time in the future, i.e. will be stored, or that must be transported in a distribution network where recontamination is a possibility, is not effectively treated (Cheremisinoff 2001). Pathogens situated in corners are not necessarily exposed to the changes in temperature and could survive this disinfecting method. Other drawbacks to thermal disinfection include the fact that it is energy intensive and therefore expensive (Kim, Anderson et al. 2002). Thermal disinfection, when applied incorrectly, will lead to rapid growth of pathogens due to the creation of ideal biotic growth conditions (Kusnetsov, Iivanainen et al. 2001). For heating disinfection to be effective, the complete water system needs to be flushed regularly to compensate for the lack of a disinfecting residual.

2.4.2.3 Chemical disinfection

The largest proportion of disinfectants used on the market can be classified as chemical in nature. Chemical disinfectants refer to all the disinfectants that trigger some chemical change or make use of a chemical concept to deactivate and destroy pathogens. The three main chemical agents are metallic ions, oxidising agents and non-oxidising agents (Kim, Anderson et al. 2002). These reactions overlap and affect each other making the actual disinfecting mechanisms difficult to identify. The formation of chemical phenomena, such as radicals, are difficult to prove but have a prominent influence on chemical disinfection (Singer, Reckhow 1999, Blatchley, Isaac 1991).

Different metals are known to have biocidal properties, but copper and silver are the most popular metallic disinfectants. The ability of silver and copper to function as disinfectants has have traditional knowledge for thousands of years used primarily for water treatment and medical applications (Alexander 2009, Klasen 2000, Silver 2003). The theory of metallic disinfection explains that the ions interfere with the cellular respiration and cellular activities by affecting the enzymes and DNA of the cell (Lin, Vidic et al. 1996, Kim, Anderson et al. 2002). The use of copper-silver ionisation as a disinfectant for drinking water has grown in popularity over the last decade (Cachafeiro, Naveira et al. 2007). Metallic ions have proved more effective than alternative disinfectants for specific situations, e.g. copper and silver ions are more effective than thermal disinfection in controlling *legionella* (Kim, Anderson et al. 2002). Metallic ions, their disinfecting ability, and disinfecting mechanisms, are discussed in detail in section *2.4.5 Metal ions*. Copper, silver, and zinc ions, as well as the use of a combination of metal ions as disinfectants are discussed.

Oxidation is a relatively simple chemical process which involves the transfer of electrons from one atom or molecule to another. The majority of South Africa's water disinfection processes make use of an oxidation process which results in oxidising agents oxidising pathogens and destroying them (Kim, Anderson et al. 2002). The main oxidising agents are halogens, chloramines, ozone, hydrogen peroxide, chlorine dioxide and potassium permanganate. There are also a few halogen-releasing organics, such as BCDMH, that have become popular lately (Kim, Anderson et al. 2002). The main advantage of oxidising agents is that many of them have a residual that keep the water clean. Oxidation is explained in full in section 2.4.4 Oxidising agents, different oxidation processes are discussed, and special emphasis is put on the use of chlorine, bromine and BCDMH as disinfectants.

There are numerous disinfecting agents that make use of a chemical characteristic, but which does not make use of metallic ions or oxidation. These disinfecting agents are referred to as non-oxidising agents. Non-oxidising agents include amines, halogenated amides, heterocyclic ketones, halogenated glycols, guanidine's, thiocarbamates, thiocyanates, aldehydes, and organo-tin compounds (Kim,

Anderson et al. 2002). These biocides are used in numerous anti-microbes and reflect a diverse efficiency range as disinfectants. The mechanism of disinfection is poorly understood for many of these non-oxidising disinfectants.

2.4.2.4 Electro-chemical disinfection

Electro-chemical disinfection refers to the use of an electro-chemical cell to trigger disinfection. Research has been done about the implementation of a variety of electro-chemical cells with different electrodes and their efficiency as disinfecting technology (Gusmão, Moraes et al. 2010). Many of the electrodes that have been tested are not practical and/or financially sustainable solutions for water disinfection. The identified technologies that are currently used for disinfection have been divided into two main categories, namely electrolytic cells, which cause the formation of an oxidising agent, and electrolytic cells, that release metallic ions. With oxidation and reduction taking place at the anode and cathode of any electro-chemical cell, it is difficult to distinguish what the actual disinfection mechanism is and what redox reactions take place. The disinfection mechanisms have been classified into oxidation through the formation of ozone, free chlorine or other oxidisers, and the biocidal effect of metallic ions.

The electrolytic cell causes two half reactions that take place at the two electrodes, oxidation (loss of electrons) takes place at the anode and reduction (gain of electrons) take place at the cathode. Depending on the voltage, water composition and electrode composition, different half reactions take place. The most common electro-chemical cells that are available on the market make use of oxide-coated electrodes and are also known as Dimensionally Stable Anodes (DSA). The idea is to have an electrolytic system that does not use the electrodes, but that the released electrons trigger a cycle of half reactions using the oxide coatings. The complexity of the reactions differs, but often concludes with the formation of the oxide layer on the anodes completing the cycle (Gusmão, Moraes et al. 2010). The formation of radicals and positively or negatively charged ions often deactivate bacteria and disinfect the water amid the cycle of reactions that are occurring (Pavlović, Pavlović et al. 2014).

A simple electrolytic setup usually disinfects water through the formation of free chlorine, ozone, peroxide, or oxygen. If there is any dissolved sodium chloride (NaCl) or other source for chlorine ions (Cl^-) in the water, then chlorine will be oxidised at the anode which will lead to the formation of free chlorine as the chlorine reacts with the water molecule (H_2O) (Singer, Reckhow 1999, Kraft 2008). The free chlorine acts as the biocide. The use of electrolysed oxidising water (EOW) or “activated water” is modern technology that results in the formation of strong oxidisers at the anode and hydroxyl ions at the cathode that are both biocidal (Badruzzaman, Khan 2002, Meireles, Giaouris et al. 2016). According to research done by Kraft on electro-chemical disinfection technologies, systems that made

use of platinum (Pt) and titanium electrodes proved to have the longest life spans (Kraft 2008). Kraft specifically investigated the technology of G.E.R.U.S. namely the 'Hypocell B4' and 'AQUADES-EL', developed by AquaRotter. Research by Kerwick, Reddy et al. (2005), however, showed that electro-chemical cells do not necessarily need the formation of chlorination species for disinfection (Kerwick, Reddy et al. 2005, Zinkevich, Beech et al. 2000).

Electro-chemical systems can also be used to produce other oxidisers. On-site formation of oxidisers through an electrolytic cell decrease transportation cost and hazards of storing chemicals (Kiuru, Sievänen et al. 2011, Martínez-Huitle, Brillas 2008). The pure electrolytic cell produces oxygen which functions as a biocide for anaerobic bacteria (Kraft 2008). Ozone can be formed at special anodes and be used as disinfectant. These special anodes make use of a diamond anode/ solid polymer electrolyte (SPE)/ cathode sandwich system such as Nafion (Kraft 2008). Graphite cathodes have proved the most efficient to produce hydrogen peroxide (H_2O_2) with higher amounts of dissolved oxygen in the water (Kraft 2008). Porous cathodes have also been developed to increase the oxygen present and support peroxide formation. Some researchers are supporting a concept of electro-sorption and direct electron transfer from the cathode to micro-organisms, as the disinfecting mechanism (Gusmão, Moraes et al. 2010, Jeong, Kim et al. 2007). Studies on the formation of OH radicals support theories that electrolytic cells could be triggering the formation of OH radicals which then act as biocide (Feng, Suzuki et al. 2004, Vega-Mercado, Martin-Belloso et al. 1997, Ohshima, Sato et al. 1997, Diao, Li et al. 2004).

When electro-chemical cells are designed correctly, the electric current causes the break-up of the electrode. An ioniser is such a special type of electro-chemical cell with a metal cathode that releases metal ions. Silver and copper-silver ionisation have grown in popularity over the last decade, commonly used in cooling towers, swimming pools, and small house-hold installations (Pavlović, Pavlović et al. 2014, Kusnetsov, Iivanainen et al. 2001, Cachafeiro, Naveira et al. 2007). The mode of disinfection is believed to be the same as using metal salts or pure metals, but the metal ions are activated and released by an electric current. The metals used for disinfection differ, but combinations used include silver, silver-copper, copper-zinc, and silver-copper-zinc. NASA was one of the technology leaders to use silver-copper ionisation for water disinfection on its spaceships and now similar technology is implemented by numerous water bottling companies. The use of electro-chemical technology leads to the reduction in water usage due to bleeding-off and financial gains due to less disinfecting chemicals used (Becker, Cohen et al. 2009).

2.4.3 Factors that influence disinfection

A few factors govern the effectiveness of disinfection. Some factors are specific to a disinfection process, but others are more general. According to a report published by the World Health Organisation the principal factors that affect disinfection are contact time, pH, temperature, and disinfectant concentration (LeChevallier, Au 2004b). The type of micro-organism and the level of resistance the specific pathogen could have developed, also play a crucial role in the efficiency of the disinfectant (Denyer, Stewart 1998). The quality of the feed water, i.e. other water contaminants, is a potential limitation to a disinfectant. A disinfectant residual, on the other hand, will prevent recontamination which influences the life-span of treated water.

Contact time and disinfectant concentration is inter-related and often referred to as CT combined. CT is the disinfectant concentration (C) multiplied with the contact time (T) and plays an integral role in the disinfection kinetics. Disinfection modelling makes use of CT and its derivatives to predict disinfection effectiveness (LeChevallier, Au 2004b, Haas, Joffe et al. 1996). Disinfectant concentrations can be extremely high, but if there is no contact time between the pathogens and disinfectants, then the disinfectant will be ineffective (Kusnetsov, Iivanainen et al. 2001). Understanding of the flow through a water system is therefore important to facilitate the spreading of the disinfectant and force contact time.

Temperature is known to influence chemical reactions as well as biological growth. Some chemical reactions are endothermic and require a minimum activation energy to trigger the reaction. The Arrhenius equation models the effect temperature has on disinfectants, although this is not valid for all disinfectants (LeChevallier, Au 2004b). Biotic species have ideal conditions in which they reproduce and grow, when temperature is in favour of pathogens multiplying the disinfection processes need to be more efficiently to destroy all the pathogens. But at drinking water temperatures, pathogen inactivation is directly proportional to temperature increases. It has been found that virus inactivation requires two or three times longer contact time periods for a decrease in temperature of 10°C (EPA 1999b).

The pH of a solution refers to the acidity or basicity of a solution, with a pH below 7.0 being acidic, 7.0 being neutral, and a pH above 7.0 basic. The pH of the feed greatly affects chemical disinfectants, since it affects the reaction kinetics (Leopold, Freese 2009, LeChevallier, Au 2004b). Chlorine, for example, is most effective at low pH levels while bromine is effective over a wider pH (Kelley 2004, Elsmore 1994). The pH affects the disinfectant species that form, these different species can either be less or more effective as disinfectants (Leopold, Freese 2009). It is therefore important to control the pH of water using lime, sodium hydroxide or sodium carbonate (Leopold, Freese 2009). The pH of

water also affects the formation of disinfecting-by-products (DBP), with a higher pH favouring chloroform formation (EPA 1999b).

The cell structure and physiology of pathogens directly influence the biocidal potential of a disinfectant. The cell membrane, cell wall, or cytoplasm can be the target of biocidal activity (Fukuzaki 2006, Denyer, Stewart 1998). Micro-organisms tend to strengthen themselves and become resistant to disinfectants through mutations and survival of the fittest. Pathogens that attach to surfaces, need a low-nutrient intake for growth and that can encapsulate themselves, are more resistant to disinfectants (LeChevallier, Au 2004b). It has been observed that most chlorine-resistant bacteria are Gram-positive or acid-fast. A possible explanation is that Gram-positive bacteria have thicker walls than Gram-negative bacteria (LeChevallier, Au 2004a). As mentioned, disinfectants are effective due to the cell structure of pathogens, therefore mutations in the cell wall, cytoplasmic membrane, and cytoplasm can lead to resistance against disinfectants (Denyer, Stewart 1998).

The quality of water will also affect the disinfecting efficiency of a disinfectant (Haas, Joffe et al. 1996). Water with a high organic load will probably react with all the oxidising agents before the oxidising agents affect the pathogens. The presence of large physical particles also inhibits disinfection and protects micro-organisms, for example pathogens are not exposed to UV when they are in the “shade” of a particle bigger than the pathogen (Meireles, Giaouris et al. 2016, EPA 1999b). Pathogens also tend to find protection within solids in contaminated water which practically inhibits disinfection.

For secondary disinfecting abilities, a disinfectant residual is needed, but not all disinfectants form a residual. Halogens are the most effective residual forming disinfectants (Kerwick, Reddy et al. 2005). Certain disinfection procedures, such as UV, form no residual, while thermal disinfection loses its residual quickly (Meireles, Giaouris et al. 2016). All disinfectant residuals can be modelled according to the quality of the water and the rate of reaction (Haas, Joffe et al. 1996). Residual forming disinfectants, such as metallic ions and oxidising agents, have an advantage over non-residual forming disinfectants, such as UV and RO, due to the prevalence of a residual that will ensure water quality and decreases possibility for recontamination (Meireles, Giaouris et al. 2016, Kusnetsov, Iivanainen et al. 2001, Kerwick, Reddy et al. 2005).

2.4.4 Oxidising agents

2.4.4.1 An overview

Oxidation is a well understood chemical reaction which is grouped with reduction as redox reactions. Redox reactions refer to the transfer of electrons from an atom, molecule or ion to another atom, molecule, or ion (James, Copeland et al. 2004). Every redox reaction consists of two half-reactions, the

oxidation half-reaction and the reduction half-reaction. Oxidation refers to the "loss" of an electron(s) that the reductant undergoes, and reduction refers to the "gain" of an electron(s) by the oxidant (Singer, Reckhow 1999, Liao, Chen et al. 2007). The terminology can be confusing and the terms oxidising agent and reducing agent will be used to simplify explanations. In Figure 5 compound A is the reducing agent and is oxidised by compound B, the oxidising agent. When working with oxidation disinfection, the oxidising agent is the disinfectant, and oxidises the reducing agent which is the pathogen, or more specifically a structural part of the pathogen.

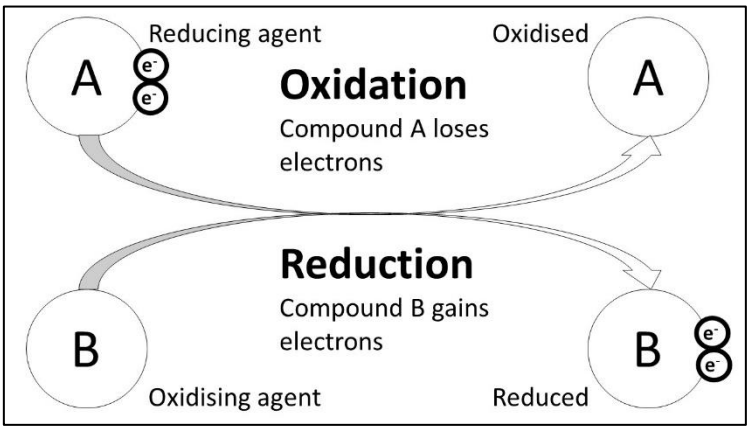

Figure 5: Visual demonstration of oxidation and reduction

Oxidising disinfectants, therefore, make use of a well understood chemical process to destabilise pathogens. Pathogens have complex structures, with some of the chemical components of the different structures more prone to be oxidised by an oxidising agent. Oxidising reactions in water can be very complex, however. The oxidation process might be followed by a variety of non-oxidising reactions including elimination-, radical chain- and hydrolysis reactions (Singer, Reckhow 1999, Blatchley, Isaac 1991). The oxidation of a part of a pathogen can lead to a variety of modes of destruction. Depending on the structure that has been damaged, some pathogens disintegrate, become self-destructive, stop multiplying, or become vulnerable to environmental conditions (Denyer, Stewart 1998). Halogens, and specifically chlorine, is believed to attack and disrupt enzyme actions of pathogens which lead to pathogen inactivation (Green, Stumpf 1946).

Halogens form the basis for most oxidising disinfectants due to their reactivity and ability to oxidise pathogens. Halogens are situated on the far right of the periodic table and easily react to gain the single electron required to fill their outermost orbital (Leopold, Freese 2009). Halogens are not often used in their pure form as disinfectants due to their instability, but is usually implemented in a molecule form that is more stable. For water disinfection, these halogen-containing-molecules usually first react with water to form a halogen specie that functions as the disinfectant. Certain research has, however, maintained that it is the molecules itself that functioned as biocide and not the free halogen

present (Williams, Elder et al. 1988). Chlorine, bromine, fluorine, and iodine are the halogens most commonly implemented as disinfectants (Koski, Stuart et al. 1966, Sletten 1974, Beckwith, Moser 1933, Kim, Anderson et al. 2002). While chlorine is most commonly used for water disinfection, bromine and bromine compounds have been used in pools, and iodine has been used in US spacecraft (Blatchley, Isaac 1991). Regulations are in place to protect the water user's health due to dangers of high halogen concentrations in water (Backer 2000, WHO 2003b).

Ozone (O_3) is currently becoming more popular as an alternative disinfectant to halogen-based oxidising agents. Ozone is an unstable gas that reverts to oxygen at room temperatures and is therefore used immediately after production on-site (Leopold, Freese 2009, Kim, Anderson et al. 2002). The on-site production of ozone requires expensive technology and infrastructure, but the running costs are lower as ozone can be made from dried air or pure oxygen. Ozone is a stronger oxidant than chlorine, but has no residual effect and cannot be stored and is therefore used in conjunction with other oxidants (Leopold, Freese 2009, LeChevallier, Au 2004a). The side effects of ozone and by-products formed are still unknown, but ozone leakage from ozone generators can be hazardous (WHO 2003b). Ozone has the advantage that pH and temperature do not greatly affect its disinfecting efficiency, but it decays faster at high pH and warm temperatures (Kim, Anderson et al. 2002, EPA 1999b, Vogt, Regli 1981). Some heterotrophic bacteria show stronger resistance to ozone than to chlorine (LeChevallier, Au 2004a).

Chlorine dioxide (ClO_2) is a highly soluble oxidising agent used to oxidise heavy metals, as well as inactivate pathogens (LeChevallier, Au 2004a). It functions as a highly selective oxidant because of its single-electron transfer reaction where it is reduced to chlorite (EPA 1999b). Chlorine dioxide is usually produced on-site which requires sophisticated equipment and expertise management. When implemented correctly, it has the advantages that it does not form THMs or oxidise bromide to bromate (EPA 1999b, LeChevallier, Au 2004a). The first application of chlorine dioxide was to oxidise contaminants that caused smells and tastes, such as algae (Vogt, Regli 1981). The disinfecting ability of chlorine dioxide is comparable to free chlorine, but less pH dependent, and more effective against specific viruses and protozoa. Since chlorine dioxide is more expensive, requires sophisticated equipment and expertise management, chlorine remains a more practical disinfectant (EPA 1999b, LeChevallier, Au 2004a).

There are numerous other oxidising agents on the market, these include hydrogen peroxide, potassium permanganate and peracetic acid. Most of these disinfecting agents are either too expensive, have not researched sufficiently, or are too complicated to compete with chlorine directly. Hydrogen peroxide (H_2O_2) is an oxidising agent that uses hydroxyl (OH•) ions, but with overall weaker

disinfecting abilities than ozone or chlorine (Kim, Anderson et al. 2002, Ong 2006). Depending on the pH and temperature it forms cytotoxic species that insures disinfection (Meireles, Giaouris et al. 2016). Potassium permanganate ($KMnO_4$) is easy to store, transport and apply, but is a poor biocide that requires extensive contact time (EPA 1999b). Peracetic acid is a powerful disinfectant, but its application is limited because it is expensive and there is the possibility of microbial regrowth (Kitis 2004). Generally oxidising disinfecting agents have been found to be more effective than non-oxidising disinfecting agents (Kim, Anderson et al. 2002).

2.4.4.2 Chlorine

Chlorine (Cl) is the most abundant halogen and the most widely used disinfectant (Sletten 1974, EPA 1999b, Leopold, Freese 2009). By the turn of the millennium, a century after the need for water disinfection was identified, two-thirds of all surface water treatment plants used chlorine as primary disinfectant (EPA 1999b). Chlorine is a halogen with a single valence electron and found as a diatomic chlorine (Cl_2) gas in its pure form. Chlorine is a strong oxidant and the half reaction of chlorine gas has a potential of +1.358 E° (Vanýsek 2012). Chlorine gas is relatively stable, which enables manufacturers to bottle it as liquid chlorine to be used as gas treatment. The production of chlorine gas is often through electrolysis of brine solution. This was traditionally done on a large scale at chlorine plants, but the production of chlorine gas on-site by small electro-chemical cells have gained popularity and is referred to as electrolysed oxidised water (EOW) (Leopold, Freese 2009).

Hypochlorous acid (HOCl) is the effective biocide of chlorine disinfectants. Hypochlorous acid is a weak acid (pK_a of about 7.5) that is pH dependent and dissociates into hydrogen cations (H^+) and hypochlorite anions (OCl^-), as shown in Equation 1.

$$HOCl \Leftrightarrow H^+ + OCl^- \qquad \text{(Eq. 1)}$$

Chlorine gas reacts with water to forming HOCl. Free chlorine is the term used to refer to the combination of Cl_2 gas dissolved, hypochlorous acid and hypochlorite anions (OSU 2011, Harp 2002). Chlorine reacts with ammonia to form combined chlorine species such as monochloramine. Total chlorine then refers to the combination of free chlorine and combined chlorine. HOCl is a stronger oxidant and a more efficient biocide than OCl^-, and therefore the preferred specie of free chlorine for disinfection (Leopold, Freese 2009). Figure 6 shows the speciation of free chlorine at different pH values and Figure 7 shows the different oxidation strengths of the different chlorine species. Chlorine demand is the term used to refer to the amount of free chlorine that is needed before a free chlorine

residual is formed, i.e. free chlorine needed to oxidise all the ammonia, organics and other reductant constituents (Leopold, Freese 2009, Fukuzaki 2006).

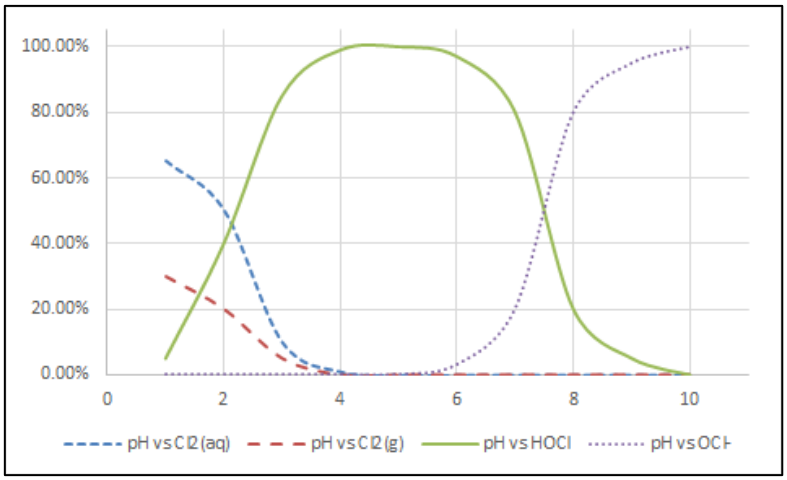

Figure 6: Chlorine speciation at different pH redrawn from Wang, Bassiri et al. (2007)

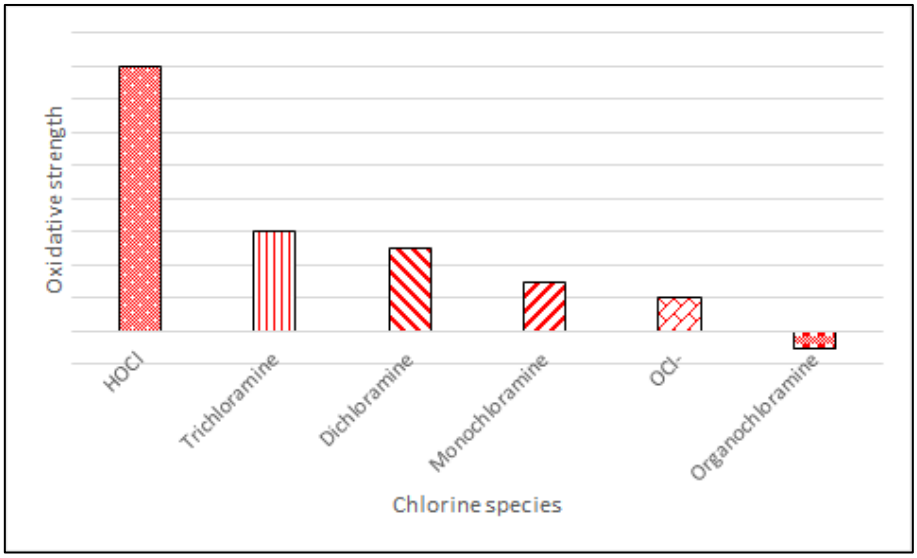

Figure 7: Oxidative strength of different chlorine species redrawn from Thomas (2006), the oxidative strength of HOCL is up to 120 times more than the oxidative strength of OCl^-

The disinfecting mode of action of free chlorine can entail a combination of reactions and the mechanisms are mostly theoretical. Research by *Oregon State University* summarises chlorine mode of action as the oxidation of free sulfhydryl groups, cell membrane and cell wall components being disrupted, and break-down of cellular macromolecules (OSU 2011). In the 1940's, Green and Stumpf showed that chlorine treatment inhibited key enzymatic processes which were irreversible. They proposed that chlorine oxidised triose-phosphoric acid (Green, Stumpf 1946, Sletten 1974). For virus inactivation, it is proposed that free chlorine attacks the amino acids in the virus capsid and the nucleic acid that is protected by the virus capsid (Shannon, Bohn et al. 2008). The lipid bilayer of the plasma

cell membrane is only penetrated by the hypochlorous acid. The hypochlorous acid can then attack the microbial cell from the inside and outside (Fukuzaki 2006).

Chlorine used for disinfection is usually used either as chlorine gas, sodium hypochlorite (NaOCl - liquid), or calcium hypochlorite ($Ca(OCl)_2$ - powder) (Leopold, Freese 2009). All three forms of chlorine react with water rapidly to form hypochlorous acid (HOCl). Equation 2 shows how chlorine gas reacts with water to form hypochlorous acid, Equation 3 shows how sodium hypochlorite reacts with water to form hypochlorous acid, and Equation 4 shows how calcium hypochlorite reacts with water to form hypochlorous acid. Chlorine gas causes a drop in pH as H^+ ions are released, while sodium hypochlorite and calcium hypochlorite cause an increase in the pH as OH^- ions are released. Although the biocidal effect of the hypochlorous acid released is the same, chlorine gas is the most efficient form of chlorine because it allows a higher percentage of HOCl to be available due to a lower pH compared to liquid and solid chlorine that causes the pH to increase (Koski, Stuart et al. 1966, Leopold, Freese 2009, Ong 2006, Singer, Reckhow 1999).

$$Cl_{2(g)} + H_2O \Longrightarrow HOCl + H^+ + Cl^- \quad \text{(Eq. 2)}$$

$$NaOCl + H_2O \Longrightarrow HOCl + Na^+ + OH^- \quad \text{(Eq. 3)}$$

$$Ca(OCl)_2 + 2H_2O \Longrightarrow 2HOCl + Ca^{2+} + 2OH^- \quad \text{(Eq. 4)}$$

When considering its global application, it is evident that chlorine has numerous advantages as disinfectant that make it popular. Primarily, free chlorine's efficiency as a water disinfectant over a wide range of common water pathogens is its biggest advantage. Chlorine treatment is easy to implement, requires limited infrastructure and has low operating costs compared to most other disinfecting technologies. Another advantage is chorine's ability to form a residual which is easily measured, and which ensures secondary disinfection (Shannon, Bohn et al. 2008). Chlorine has proved itself a reliable disinfectant over the last century with an extensive safety record in improving water quality (EPA 1999b, Leopold, Freese 2009). Other contaminants that chlorine oxidises out are organic and inorganic compounds, while odours are also often removed (EPA 1999a).

Human safety has become a major concern with the application of chlorine gas as disinfectant (Blatchley, Isaac 1991). Chlorine has numerous negative effects and negative by-products, such as trihalomethanes (e.g. chloroform), that form under certain conditions. Some of these by-products are toxic and carcinogenic (Trussell, Umphres 1978). Although chlorine residual is usually wanted in water distribution systems, it has an adverse effect on aquatic life when treated water is released back into the environment. All the forms of chlorine are highly corrosive, which tend to decrease the life-span

of distribution networks, pumps, and storage containers. Some parasitic species, especially protozoa, are very resistant to chlorine treatment, such species include *Cryptosporidium parvum*, *Mycobacterium avium*, *Entamoeba histolytica*, and *Giardia lamblia* (EPA 1999a, LeChevallier, Au 2004a, Martínez, Gallegos et al. 2004, Shannon, Bohn et al. 2008, Leopold, Freese 2009, Meireles, Giaouris et al. 2016). When combined in multiple-barrier processes, chlorination also showed fouling on membranes and the oxidation of organics lead to accelerated after-growth of planktonic and sessile bacteria (Blatchley, Isaac 1991).

It is evident that there is a need for alternative disinfectants to chlorine. Depending on the application of the disinfection, it makes sense to combine chlorine treatment with alternative treatments to limit the disadvantages of chlorine. In some cases, chemical usage needs to be minimised, and the combination of chlorine with UV is an efficient solution. The addition of chlorine with ammonia causes the formation of chloramines. Chloramines are weaker biocides than free chlorine, but have a longer half-life and produce less hazardous by-products (Leopold, Freese 2009). Chlorine is also used in conjunction with bromine and iodine in swimming pool applications (Koski, Stuart et al. 1966, EPA 1999b). Chlorine dioxide is implemented similarly to chloramines, it is produced from chlorine gas, but used as alternative disinfectant because it is less hazardous than chlorine (Kim, Anderson et al. 2002).

2.4.4.3 Bromine

Bromine is comparable to chlorine, in that it is a halogen with a single valence electron and forms diatomic bromine (Br_2) in its pure form. Bromine is a reddish-brown liquid at room temperature, but dangerous and toxic to handle (Leopold, Freese 2009). The first recorded uses of bromine as disinfectant is in the 1930's (Nalepa 2004). Liquid bromine and bromate ions (BrO_3^-) are extremely unstable in acid solutions, reacting with water (H_2O) to form hypobromous acid (Betts, Mackenzie 1951). Hypobromous acid is a weak acid (pK_a of about 8.8) that is less pH dependent than hypochlorous acid. Hypobromous acid (HOBr) is the active disinfectant, and dissociates to hydrogen cations (H^+) and hypobromite anions (OBr^-), seen in Equation 5 (Kim, Anderson et al. 2002). Bromine is therefore a reliable disinfectant over a broader pH range than chlorine (Williams, Bridges 2010, WHO 2016). Figure 8 depicts the speciation of bromine at different pH.

$$HOBr \Leftrightarrow H^+ + OBr^- \quad \text{(Eq. 5)}$$

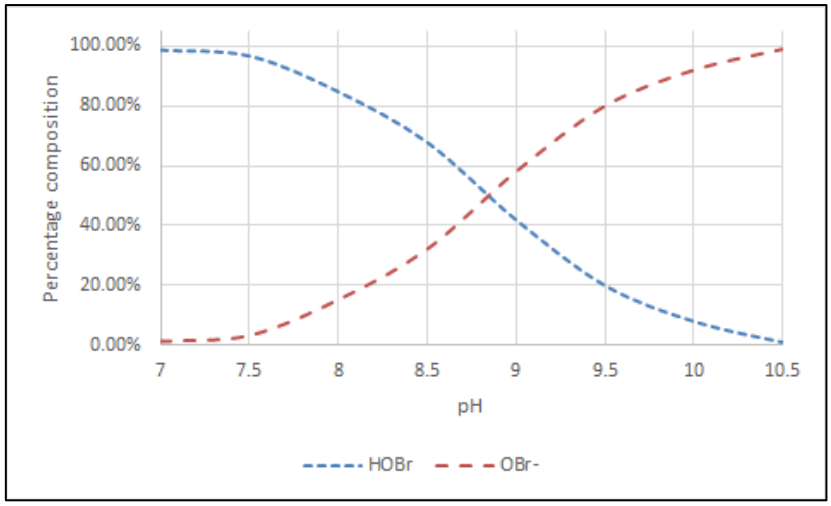

Figure 8: Bromine speciation at different pH from Health Canada (2015)

Bromine liquid is the strongest oxidant of all the bromine forms and react with water to form hypobromous acid, Equation 6 (Koski, Stuart et al. 1966). But, due to the hazards of working with liquid bromine, bromine salts, such as sodium bromide (NaBr), are more frequently used. These salts require an oxidant to release the bromine and form hypobromous acid, depicted by Equation 7 (Elsmore 1994). Free bromine and combined bromine are both effective disinfectants and therefore only a "total" bromine is measured when disinfectant residual needs to be monitored (LeChevallier, Au 2004a). Bromine releasing organics, such as BCDMH, and N-bromamines, are other popular alternatives to bromine liquid (Williams, Elder et al. 1988). The disinfection mechanism is explained by the hypobromous acid combining with the protoplasm of the micro-organisms to form nitrogen-bromine bonds that disrupt the metabolic process (Walker, Rogers et al. 1994, WHO 2016).

$$Br_{2(l)} + H_2O \Longrightarrow HOBr + H^+ + Br^- \quad \text{(Eq. 6)}$$

$$NaBr + HOCl \Leftrightarrow HOBr + Na^+ + Cl^- \quad \text{(Eq. 7)}$$

The main factors that influence bromine disinfection is temperature, bromine concentration, contact time, pH and organic content (Elsmore 1994). A recent study by the World Health Organisation reported the resistance of organisms to bromine to be in the following order, with decreasing resistance (WHO 2016):

bacterial spores > mould spores > yeasts and non-spore-forming bacteria

The main advantage of bromine is its efficiency over a wider pH range and against pathogens that are resistant to chlorine (Walker, Rogers et al. 1994). For example, the protozoan cysts of the parasite *Entamoeba histolytica* show less resistance to bromine than to chlorine or iodine (WHO 2016, Kim

2014). Bromine is commonly referred to as the strongest disinfectant of all the halogen-based disinfectants (Beckwith, Moser 1933, Koski, Stuart et al. 1966). Bromine is a better oxidising agent than similar chlorine species, but has a lower residual (Leopold, Freese 2009). Biofilm containing bacteria is also more easily inactivated by bromine disinfectants compared to other oxidisers (Williams, Bridges 2010). Bromine is implemented as disinfectant in nature by stationary organisms such as seaweeds, sponges, and bryozoans to prevent fouling of bacteria, fungi, and algae (Nalepa 2004).

The application of bromine disinfection for potable water is limited due to the belief that bromine is carcinogenic, and water treated with bromine usually has a bad taste and odour. However, the International Agency for Research on Cancer (IARC) does not have bromide or bromate on their list of carcinogenic agents (WHO 2016). Human exposure to large doses of bromide cause nausea, vomiting, abdominal pain, and, under extreme conditions, coma and paralysis (Kim 2014, WHO 2009). Free bromine also reacts with natural organic matter (NOM) to form by-products such as THMs which are carcinogenic (EPA 1999b, Leopold, Freese 2009, Kim, Anderson et al. 2002, Singer, Reckhow 1999, Meireles, Giaouris et al. 2016). As with chlorine, certain pathogens, such as *Cryptosporidium,* show resistance to bromine treatment. Outdoor use of bromine disinfection is limited as bromine residual is rapidly broken-up by sunlight (LeChevallier, Au 2004a). Since bromine is more expensive than chlorine, it remains an unpopular alternative (Nalepa 2004).

Bromine's strong biocidal ability has led to it being used in numerous non-drinking water utilities such as swimming pools, wastewater works, and cooling towers. Bromine's efficiency against *legionella* has made it a popular disinfectant in cooling towers (Kim, Anderson et al. 2002). Traditionally, bromine salts and bromine liquid have not really been implemented to treat potable water, although it is now being used on USA Navy ships, oil rigs and even commercial ships (Cortruvo 2015, Hatch, Korslin 2003). When used for drinking water, the bromine, its taste and smell is stripped by granular activated carbon (GAC) (Kim, Anderson et al. 2002, Leopold, Freese 2009, Thompson, Megonnell 2003). The application of bromine with other disinfectants are also common. B-halamines form when bromine is combined with ammonia, which is more efficient than chlorine combined with ammonia (Hatch, Korslin 2003, WHO 2016). The addition of bromide to chlorine solutions have also showed improved biocidal activity (Nalepa 2004).

2.4.4.4 Bromo-chloro-dimethyl-hydantoin (BCDMH)

Bromo-chloro-dimethyl-hydantoin (BCDMH) is an organic compound that releases bromine and chlorine when reacting with water. The molecular formula for BCDMH is $C_5H_6BrClN_2O_2$ and the structural formula can be seen in Figure 9. BCDMH is nearly insoluble, with a solubility of 15% (w/v) at

20°C and 20% (w/v) at 25°C (Walker, Rogers et al. 1994). The chemical was patented in 1957, but was used as disinfectant in a cooling system for the first time in the 1970s (Nalepa 2004). BCDMH is commercially available as a white tablet or powder, while the production of a gel BCDMH is under investigation (Envirotech 1995, Elsmore 1994, Kim, Anderson et al. 2002).

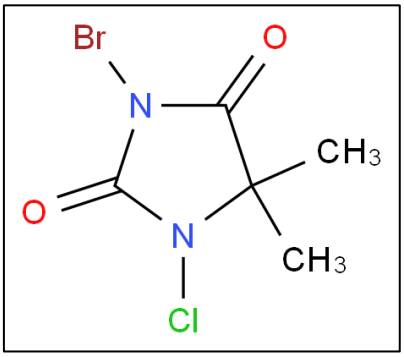

Figure 9: 2D structure of BCDMH

The mode of disinfection is a combination of that of bromine and chlorine. Hypobromous acid (HOBr) and hypochlorous acid (HOCl) form as BCDMH react with water. The formation of hypobromous acid and hypochlorous acid is separated as different equations, because the formation of hypobromous acid is fast and the formation of hypochlorous acid is slow (Equation 8 and Equation 9) (Nalepa 2004). When bromide ions (Br^-) form from oxidation reactions, or the dissociation of the hypobromite ions (OBr^-), the bromide ion reacts with hypochlorous acid to form hypobromous acid and a chloride ion (Cl^-) (Equation 10). The result is that the reactions favour hypobromous acid (HOBr) over hypochlorous acid (HOCl) and therefore hypobromous acid is usually the active disinfectant (Envirotech 1995, Moffa, Davis et al. 2006).

$$BCDMH + H_2O \Longrightarrow CDMH + HOBr \text{ (Fast reaction)} \quad \text{(Eq. 8)}$$

$$CDMH + H_2O \Longrightarrow DMH + HOCl \text{ (Slow reaction)} \quad \text{(Eq. 9)}$$

$$Br^- + HOCl \Longrightarrow HOBr + Cl^- \quad \text{(Eq. 10)}$$

With:

- BCDMH = bromo-chloro-dimethyl-hydantoin = $C_5H_6BrClN_2O_2$
- CDMH = chloro-dimethyl-hydantoin = $C_5H_7ClN_2O_2$
- DMH = dimethyl-hydantoin = $C_5H_8N_2O_2$

Research done by Walker showed that BCDMH concentration and ORP show a strong correlation up to a BCDMH concentration of 3-4 mg/l which correspond with an ORP of 300 to 400 mV. Possibly due

to the bromine component, *legionella* is more prone to be activated by BCDMH than other cooling tower bacteria. As expected, sessile bacteria in biofilms are more resistant to BCDMH than planktonic bacteria (Walker, Rogers et al. 1994). The contact time associated with BCDMH treatment is short, a few minutes is usually sufficient (Takahashi, Kirihara et al. 2005, Howarth 2010, Kim, Anderson et al. 2002).

The advantages of BCDMH as disinfectant is a combination of its chemical and physical properties. Chemically, BCDMH show biocidal efficiency against a wide spectrum of pathogens and is effective in a variety of water conditions. Its bromine content makes it efficient in acidic and basic water, and causes it to remain biocidal in the presence of ammonia with the formation of bromamines (McCoy, Wireman 1989). BCDMH does not show strong corrosive characteristics at recommended treatment concentrations, but will corrode most metals at high concentrations. Physically, the tablet and powder form of BCDMH is easy to work with and easy to store. If not exposed to moisture, it has a long shelf-life. Its low solubility causes the reaction with water to be slow and controlled which leads to higher efficient treatment and better control. To implement BCDMH treatment, no sophisticated equipment is need and no large footprint either (Takahashi, Kirihara et al. 2005, EPA 2013, Soracco, Wilde et al. 1985).

The dangers of BCDMH are comparable to those of bromine and chlorine treatment, but to a lesser effect, because of lower free chlorine and lower bromine concentrations. Organic content will still cause the formation of THMs which are dangerous and believed to be carcinogenic (Elsmore 1994, Moffa, Davis et al. 2006). BCDMH is more expensive than chlorine which limits its use initially, but its other advantages make the overall cost of applied BCDMH as disinfectant financially competitive to chlorine treatment (Soracco, Wilde et al. 1985).

When compared to chlorine efficiency, at a pH of 8.5 it is a stronger biocide against *E. aerogenes*, *E. coli*, *P. aeruginosa*, and *polybacteria* (Nalepa 2004, Zhang, Matson 1989). Practical case studies that compared BCDMH application with chlorine application showed overall improvement for BCDMH as disinfectant. While BCDMH control of sessile and planktonic bacteria is more effective, less chemicals are used in BCDMH systems and pH varies less compared to chlorine systems. BCDMH applications required less corrosion inhibitor while the measured corrosion levels were less than with similar chlorine concentrations (Nalepa 2004). Moffa, Davis et al. concluded, from a pilot study, that BCDMH can give better biocidal results in less time and with lower by-products than sodium hypochlorite (Moffa, Davis et al. 2006).

2.4.5 Metal ions

2.4.5.1 An overview

The ability of silver and copper to function as disinfectants have been traditional knowledge for centuries. In the Middle Ages water was stored in copper or silver containers to keep it clean from algae and other organisms. The Vikings used copper strings on their ships to prevent the growth of algae, this concept is still used in modern ships by adding copper or silver into the paint mixtures. There are three main forms that metals are used as disinfectants; traditionally as the pure metals, more recently as metal ions released from salts or ionisation, and the newest technology releases Nano-metal particles (Lin, Vidic et al. 1996). Metals are not always biocidal, micro-organisms often require a certain amount of the different metal compounds for normal cell functionality (Cuppett, Duncan et al. 2006, WHO 2003a).

The mode of disinfection of metals is not fully understood. The difference, if any, in the mode of disinfection between pure metals, metal ions, and Nano-metals is still theoretical and difficult to prove. Metal ions are thought to disrupt enzymes that facilitate cellular respiration, and bind with specific DNA (Kim, Anderson et al. 2002). Amino acids and other thiol groups also become targets for metal ions to react with which affect cell functionality (Liau, Read et al. 1997, Lin, Vidic et al. 1996). Metallic ions are positively charged and attach to the negatively charged cell wall, destroying permeability which leads to cell death (Lin, Vidic et al. 1996, Huang, Shih et al. 2008). Metals require a substantial longer contact time for efficient disinfection compared with oxidising agents, ranging from a few hours to even days (Kim, Anderson et al. 2002, Pavlović, Pavlović et al. 2014). Depending on the constituents in the water, metal ions form different complexes with other compounds, these complexes are believed to play additional roles in disinfection (Majzlik, Strasky et al. 2011).

Metal treatment is applied in different ways. Pure metal containers or surfaces are made which is then used for storage or as working platforms for sterile work (Santo, Morais et al. 2010, Nies 1999, Cuppett, Duncan et al. 2006). The addition of metallic salts, such as silver nitrate ($AgNO_3$), releases metallic cations when dissolved in water. The electro-chemical process of ionisation is the alternative method implemented to release metal ions through the break-up of the metal electrodes (Liau, Read et al. 1997). Ionisation, unfortunately, requires periodic cleaning of the electrodes (Kim, Anderson et al. 2002). Nano metals are formed using special techniques that ensure the metal particles are extremely small, these nanoparticles are then often imbedded in other structures. More research has been done about the application of metal ions in warm water systems than in cold water systems (Kim, Anderson et al. 2002).

Metals are toxic to most life forms, including humans, in high enough concentrations. Heavy metals are toxic at very small concentrations, while other metals become toxic at higher concentrations (Majzlik, Strasky et al. 2011). When using metals for treatment it is necessary to maintain concentrations far below human toxic levels to ensure water user safety and to protect the environment (Nies 1999). Metal ions, or specifically heavy metal ions, are often part of the contaminants that need to be oxidised out (EPA 1999b). Micro-organism mutations have been detected that are heavy metal resistant and not susceptible to toxic metals (Majzlik, Strasky et al. 2011, Silver, Phung 1996). The biocidal abilities of metals are rated as follow: Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn (Kim, Kuk et al. 2007).

2.4.5.2 Copper

Copper (Cu) is a transition metal that is soft and ductile and used in a variety of appliances because of its excellent thermal and electrical conductivity. Copper atoms can lose a single or both its valence electrons to form copper ions (Cu^{+} or Cu^{2+}) during redox reactions. The ionic form of copper (II) ions (Cu^{2+}) is more stable and more common than copper (I) ions. The half reactions and their standard potentials are given below in Equation 11, Equation 12 and Equation 13. In pure water, copper has a solubility of 1 mg/L at a pH of 7 (Kim, Anderson et al. 2002).

$$Cu^{2+} + e^{-} \Leftrightarrow Cu^{+} \text{ (+0.159 E°) (Vanýsek 2012)} \quad \text{(Eq. 11)}$$

$$Cu^{2+} + 2e^{-} \Leftrightarrow Cu_{(s)} \text{ (+0.337 E°) (Vanýsek 2012)} \quad \text{(Eq. 12)}$$

$$Cu^{+} + e^{-} \Leftrightarrow Cu_{(s)} \text{ (+0.520 E°) (Vanýsek 2012)} \quad \text{(Eq. 13)}$$

Copper has been implemented as a disinfectant in metal surfaces such as copper water jugs, door knobs, and coins for centuries (Santo, Morais et al. 2010). Copper inactivates a wide variety of pathogens, from yeast to bacteria, including *E. coli* (Kejdusova, Vyslouzil et al. 2015, Martínez, Gallegos et al. 2004). Copper salts and ionised copper show anti-bacterial characteristics from concentrations as low as 0.1 mg/L up to 0.8 mg/L, but require at least a few hours contact time to ensure disinfection (Huang, Shih et al. 2008). Water treatment by copper is practical because the copper concentrations required is less than the solubility of copper and is below the maximum concentration level goals (MCLG) for copper, of 1.3 mg/L, prescribed by the EPA (Kim, Anderson et al. 2002). The combination of copper with other molecules have been researched, for example copper (II) ions cross-linked with Carmellose (CMC) is used for a slow release of the metal biocide (Kejdusova, Vyslouzil et al. 2015).

The anti-microbial mode of action for copper is a combination of the copper interacting with the pathogenic cell, but also the formation of other copper complexes. Fewtrell (2014) explains the copper

ions are positively charged and form electrostatic compounds with negatively charged cell walls of micro-organisms which disturb cell wall permeability and the nutrient uptake of micro-organisms (Fewtrell 2014). Nies (1993) explains that copper toxicity comes from the production of hyperoxide radicals and the ensuing interaction with the cell membrane. A variety of redox reaction take place within copper disinfection depending on the availability of molecular oxygen and other radicals (Nies 1999).

As some other metals, copper is an essential nutrient for cell functionality, but at small concentrations (Cuppett, Duncan et al. 2006, Becerra-Castro, Machado et al. 2015). It has been found that microbes develop resistance to dry copper more easily than to copper ions in solution (Santo, Morais et al. 2010). The tasting detection limit for copper has been researched, but results were not coherent. It was determined that soluble copper species were more readily tasted than particulate copper. A copper taste was detectable at concentrations from 0.4 to 0.8 mg/L, which is still below the concentrations that could cause health issues (Cuppett, Duncan et al. 2006). Copper ions can be removed from solutions by precipitation or sorption by organic solids, clays, or minerals (Santo, Morais et al. 2010).

2.4.5.3 Silver

Silver (Ag) is an expensive metal that is an excellent thermal and electrical conductor. Silver can be oxidised to silver ions (Ag^+ or Ag^{2+}) when exposed to strong oxidising agents. The half reactions and corresponding standard potentials are given in Equation 14 and Equation 15 for the formation of silver. Silver has become a popular alternative to chlorine disinfection.

$$Ag^+ + e^- \Leftrightarrow Ag_{(s)} \text{ (+0.7796 E°) (Vanýsek 2012)} \quad \text{(Eq. 14)}$$

$$Ag^{2+} + e^- \Leftrightarrow Ag^+ \text{ (+1.98 E°) (Vanýsek 2012)} \quad \text{(Eq. 15)}$$

Silver is the best known and most implemented metal biocide (Pavlović, Pavlović et al. 2014). Different studies have shown silver as being an effective biocide over a wide variety of bacteria, fungi, and viruses, and not only water pathogens. Investigations into silver applications showed that the contact time requirements are relatively long, with minimum a few hours and sometimes days required for disinfection (Fewtrell 2014, Jung, Koo et al. 2008). Silver has been used for a long time in wound-dressings and other medicinal applications (Nies 1999, Rai, Yadav et al. 2009). For disinfection, silver is usually implemented as silver ions (Ag^+) or silver nanoparticles (NP) (Rai, Yadav et al. 2009, Greulich, Braun et al. 2012).

Silver ions are produced through the introduction of silver salts, $AgNO_3$ or AgCl, or through an electrolytic cell with silver electrodes (Fewtrell 2014). Silver ion concentrations required for disinfection varied according to different experiments and corresponding controls. Huang, Shih et al. (2008) found that silver only becomes biocidal at concentrations above 0.08 mg/L (Huang, Shih et al. 2008). The biocidal properties of pure silver metal are understood to be more related to silver ions acting as disinfectant than the pure metal functioning as disinfectant. Silver oxidises in low pH environments and in the presence of oxidants, such as dissolved oxygen, into silver ions which act as biocide (Dowling, Betts et al. 2003). The bacteria *E. coli*, for example, has an acidic pH and oxidising membrane which will catalyse the release of silver ions (López-Heras, Theodorou et al. 2015).

The mechanisms of silver disinfection are complex, but a combination of theories can be compiled. Bragg and Rainnie (1974), writes that silver ions react with the respiratory chain at two levels. The silver ions inhibit substrate oxidation of cells which could affect substrate transport, substrate metabolism and the respiratory chain itself. For the disinfection of *E. coli* for example, silver has been seen to prevent the oxidation which the intact cell suspensions of *E. coli* usually do, these include the oxidation of glucose, glycerol, fumarate, succinate, D- and L-lactate, and endogenous substrates. The cell becomes self-destructive and unable to function normally (Bragg, Rainnie 1974). It has also been proposed that the disinfecting mechanism of silver includes silver's reaction with the thiol groups, amino acid being a member of the thiol group, but also present in the cell structure of many pathogens (Liau, Read et al. 1997, Mulley, Jenkins et al. 2014). Proteins in the cell wall interact with the silver ions which lead to the disruption of the proteins and accumulation of silver ions in the cell wall. Silver ions also destabilise the cell membrane, cause the formation of pits in the cell walls and membranes, which lead to an increased permeability to silver ions and other disinfectants (López-Heras, Theodorou et al. 2015, Li, Xie et al. 2010). *Jung, Koo et al.* proposed that silver ions caused bacteria to go into an "active but nonculturable" (ABNC) state (Jung, Koo et al. 2008).

The chemical reduction of a silver salt, such as silver nitrate ($AgNO_3$), is often used to produces silver nanoparticles. Other methods of producing nanoparticles include electrochemical reduction, cytochemical synthesis, solution radiation, and spark discharging (Fewtrell 2014). Silver nanoparticles are believed to function as disinfectant directly, but also to cause the release of silver ions (Zhang, Yao et al. 2011). One of the main advantages of silver nanoparticles is an increased surface area compared to other forms of silver, which quicken exposure of pathogens to silver, and cause a higher oxidation rate of silver into silver ions (López-Heras, Theodorou et al. 2015). The disinfecting agents are believed to be a combination of the silver nanoparticles themselves and the formation of silver ions that act as disinfectants. Silver nanoparticles can be imbedded in other technology, such as root canal treatment

(Krishnan, Arumugam et al. 2015). Silver nanoparticles show higher efficiency as disinfectant at higher temperatures, up to 37 °C, and in aerobic conditions, i.e. when exposed to oxygen (Xu, Qu et al. 2012).

A major advantage of silver, and other metal disinfectants, is the absence of any disinfection by-products (DBP) (Fewtrell 2014). When compared to other disinfectants, there are fewer pathogens that are resistant to silver and fewer mutation strains that are resistant. According to Kim, Kuk et al. (2007), the toxicity of silver is low against mammalian cells compared to its toxicity against micro-organisms (Kim, Kuk et al. 2007). Silver can be applied in such a wide variety of ways from coatings, slow release molecules to ionisation that the safety hazards for application and storage of silver are minimal (Rai, Yadav et al. 2009).

The dangers of silver disinfection have been mainly related to the unknown long-term effects it may have on humans and the concentrations in which it will damage healthy cells (López-Heras, Theodorou et al. 2015). Greulich, Braun et al. (2012) has claimed that silver nanoparticles and silver ions cause cell damage at similar concentrations at which it inactivates micro-organisms (Greulich, Braun et al. 2012). There has been concerns that silver nanoparticles might pass through the brain membrane and damage brain cells. The WHO currently has a life time health advisory concentration of 0.1 mg/L on silver intake (Kim, Anderson et al. 2002). Large scale use of silver as disinfectant poses the possible danger of a wide range of silver resistant pathogens developing (Silver 2003).

2.4.5.4 Zinc

Zinc is a metal with a wide variety of known uses, but not a common disinfectant or water disinfectant. Chemically, zinc can be oxidised to form zinc ions (Zn^{2+}), the half reaction of zinc and standard potential is given in Equation 16. The role zinc plays as water disinfectant is two sided, because too little zinc can cause growth inhibition and too much zinc can be toxic. Since zinc forms a variety of molecules that are components in enzymes and proteins, the removal of zinc often leads to inactivation of micro-organisms (Nies 1999). Although zinc is essential for micro-organisms to live, at high concentrations, zinc seemingly becomes toxic or have been seen to exert selective pressures on bacteria (Becerra-Castro, Machado et al. 2015).

$$Zn^{2+} + 2e^{-} \Leftrightarrow Zn_{(s)} \text{ (-0.7618 E°) (Vanýsek 2012)} \qquad \text{(Eq. 16)}$$

Zinc has also been identified as one of the heavy metals with possible environmental concerns. As with silver and copper, zinc bioaccumulates in cells. Cell enzymes and respiratory activities are affected by the increase in zinc concentration until the cell functionality is damaged (Dang, Doan et al. 2009). There is indirect evidence that zinc ions, like silver ions, inhibit the respiratory chain of living cells (Bragg, Rainnie 1974). Zinc is, however, the weakest biocide when compared to silver and copper

(Kim, Kuk et al. 2007). Some studies have investigated zinc salts and possible benefits over copper salts. Becerra-Castro, Machado et al. (2015) reports that zinc chloride ($ZnCl_2$) showed stronger bacterial growth inhibitory characteristics than cupric sulphate salt ($CuSO_4 \bullet 5H_2O$) (Becerra-Castro, Machado et al. 2015).

2.4.5.5 Metal combinations

The combination of different metals for water disinfection has been investigated and implemented for a couple of decades. As mentioned earlier, copper and silver are metals that have been used as water disinfectants for ages. The combination of silver and copper was therefore not an abnormal idea. Since the introduction of copper and silver combined treatment, various publications have found a synergistic effect of the metals combined (Kim, Anderson et al. 2002, Huang, Shih et al. 2008). The use of copper and silver ions through copper-silver ionisation has grown in popularity over the last decade (Zheng, Dunets et al. 2012, Cachafeiro, Naveira et al. 2007). It was however only in 2008 that metallic alloys were registered as disinfectants (Santo, Morais et al. 2010).

A plausible explanation of the combined disinfection mechanism of copper and silver ions is the following. Copper ions are positively charged and form electrostatic compounds with negatively charged cell walls of micro-organisms which disturb cell wall permeability and nutrient uptake of micro-organisms (Fewtrell 2014). After the copper ions penetrate the cell wall, the negative silver ions interact with the interior of the cells and make the cells non-functional (Lin, Vidic et al. 1996). When treating copper and silver ionisation it seems there is a limited amount of metal ions required to cause disinfection, and an excess of metal ions will not increase the rate of disinfection (Lin, Vidic et al. 1996).

Copper-silver ionisation is used in various different systems and often instead of chlorine or in combination with chlorine (Cassells, Yahya et al. 1995, Fewtrell 2014). Copper-silver disinfection systems are used in swimming pools, cooling towers and have been very effective to destroy *legionella* in hospital water systems (Kusnetsov, Iivanainen et al. 2001). NASA started using copper-silver ionisation to provide drinking water on spaceships and this has led to numerous water bottling companies making use of similar technology to provide potable water (Carefree Clearwater 2015). Most copper-silver treatment systems use a copper silver ratio of ten-parts copper to one-part silver (10 copper: 1 silver), at concentrations below the EPA limits (Liu, Stout et al. 1994).

Silver-copper ionisation is the only disinfectant to have complied with a list of criteria to ensure efficiency in treating *legionella* over a 5-year study (Stout, Victor 2003). On the synergism of copper-silver treatment, different studies have been conducted. Liu, Stout et al. (1998) did in-vitro experiments that showed copper and silver ions act synergistically to kill *legionella* (Liu, Stout et al. 1998). Kusnetsov, Iivanainen et al. (2001) investigated silver-copper ionisation in warm water systems,

they eradicated *legionella*, but did not discuss any synergistic relationships (Kusnetsov, Iivanainen et al. 2001). Huang, Shih et al. (2008) did an extended investigation in 300 hospitals in the USA and reported that copper and silver showed synergy in treating *P. aeruginosa* and *A. baumannii*. But that the combination exhibited an unwanted effect against *S. maltophilia* (Huang, Shih et al. 2008). Shih and Lin *(2010)* reported that below EPA limits, copper-silver ionisation can control *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* in biofilms and in planktonic phases (Shih, Lin 2010). Cachafeiro, Naveira et al. (2007) discussed the effect of temperature, and mentioned that high temperatures increase effectiveness of copper-silver disinfection (Cachafeiro, Naveira et al. 2007).

Combining different metals in a single treatment procedure have several advantages. The advantages include that no chemicals need to be made, stored, transported, handled, or discarded (Zheng, Dunets et al. 2012, Liu, Stout et al. 1994). There is little maintenance on an ioniser except for the cleaning and replacing of the electrodes (Zheng, Dunets et al. 2012, Liu, Stout et al. 1994). Copper and silver ionisation have proved to have a good residual effect as the metals accumulate at the bottom of storage containers (Kim, Anderson et al. 2002, Zheng, Dunets et al. 2012, Liu, Stout et al. 1994). High or low organic content does not affect disinfection efficiency (Fisher, Burton et al. 1999). Zinc, copper, and silver are all divided into three different groups with different resistant mechanisms that need to develop in pathogens and therefore decrease probability of resistance developing to all three metals (Chudobova, Dostalova et al. 2015). There are no known by-products that form from metallic treatment and it is safe whilst ion concentrations are kept under recommended levels (Liu, Stout et al. 1994, Cachafeiro, Naveira et al. 2007). Other advantages include low installation and maintenance costs as well as a simple installation procedure (Liu, Stout et al. 1994).

The disadvantages of mixed metallic treatment are still relatively unknown. Ionisation has a few technological limitations. The maintenance requires periodic cleaning and replacement of electrodes to ensure metal release (Liu, Stout et al. 1994, Kusnetsov, Iivanainen et al. 2001). Changes in water content, such as high salinity, might completely change oxidation reactions taking place and not release any metals. Different metals have different oxidation potentials, therefore there is very little control over the actual reactions taking place with regards to metal release and disinfection. The technology has not been implemented for long enough to see the long-term effects of such treatment on humans, with potable water treatment, or on the environment, with effluent treatment. Further disadvantages include the lack of data on phytotoxicity and efficacy (Zheng, Dunets et al. 2012). The release of high amounts of metal treated water into the environment must be avoided to prevent metal build-up to toxic levels (Martínez, Gallegos et al. 2004, Zheng, Dunets et al. 2012). Metal ions showed limited efficiency to disinfect when amoeba was present (Cassells, Yahya et al. 1995).

Lin, Vidic et al. (2002) investigated the effect of different water characteristics on copper-silver ionisation disinfection. They found that changes in bicarbonate ion concentrations, water hardness, and dissolved organic carbon (DOC) had no significant impact on the efficacy of silver-copper treatment. They did, however, find that an increase in pH from 7.0 to 9.0 can decrease disinfection efficiency from 1 000 000-fold to 10-fold for a 24-hour contact time. The acidity of a water source therefore effects not only chlorine disinfection, but copper-silver disinfection as well. A further observation they made was that insoluble copper complexes precipitated at a pH above 6 (Lin, Vidic et al. 2002).

2.4.6 Combination technologies

2.4.6.1 Water disinfection combinations

Water treatment is complex and can take on a wide variety of forms. Water disinfection is a specific component of water treatment, but it also remains complex. The two main contributing factors that make it so complex are the quality of the source water to be treated and the water quality required after treatment. Water quality required is often standardised, but the quality of different water sources will differ dramatically, even the water quality from a single source can vary drastically. The complexity of water treatment requires the development of tailor-made treatment processes that combine different technologies to make use of their separate strengths and have a more robust combined product (World Health Organization 2008, Schutte, Focke 2006, Cheremisinoff 2001).

Water treatment is generally a multi-barrier process, with each step removing a different contaminant. For example, filtration will be used to remove large solids, flocculation and coagulation will be used to remove suspended particles, a bioreactor will be used to remove organics, a softener will be used to remove calcium carbonate and a disinfectant will be used to remove pathogens (Schutte, Focke 2006, Cheremisinoff 2001). Similarly, different water disinfectants can be combined to cover a wider range of pathogens or disinfectants can be combined with other treatment technology that improves the disinfectant's efficiency. When disinfectants are combined with activated carbon filters, for example, the activated carbon remove excess disinfectant, taste and colour, making the water more aesthetical (Thompson, Megonnell 2003).

The combination of different disinfecting technologies can have three main combined effects as disinfectants. Firstly, the combined technology can interfere with each other and cause an overall decrease in disinfection. The combined technology will then not be a viable treatment solution. Secondly, the combined technology can have a non-interactive addition effect on each other which makes the final effectiveness the same as the effectiveness of the individual processes combined. Such a combination could have advantages in decreasing risks and increasing water quality. Thirdly, the

combined technology can have a synergistic interaction. The individual processes then strengthen each other to have a higher efficiency than what would be seen by adding the disinfecting efficiency only.

Disinfectants have their strengths and weaknesses and therefore disinfection processes are combined to limit the overall disadvantages of the system (Shannon, Bohn et al. 2008, Meireles, Giaouris et al. 2016). For example, filtration, especially membrane filtration, can remove pathogens, but will be more efficient to decrease the organic content of water. By combining filtration with an oxidising disinfectant, it decreases the pressure on the oxidising agent, which means a lower disinfectant dosage is required and less by-products are formed. RO, on the other hand, removes nearly everything from water, but the essential minerals are replaced and there is no disinfectant residual, a residual-giving disinfectant must therefore be used in combination with RO treatment to ensure secondary disinfection (Schutte, Focke 2006, Cheremisinoff 2001).

Many disinfection combinations have a mere additional effect, as the one disinfectant has a specific pathogen range it deactivates and the other disinfectant might give a residual. Filtration or RO with an oxidising agent functions like this. Other combinations combine UV with chlorine and ozone with chlorine, in both cases chlorine plays more a disinfectant residual role (Liviac, Wagner et al. 2010, Shannon, Bohn et al. 2008). There has been research into the formation of other chemical phenomena with such combinations, where the mode of disinfection changes. An example is ozone with hydrogen peroxide and is referred to as perozone. The hydrogen peroxide is understood to catalyse the formation of hydroxyl radicals that function as the active disinfectant (EPA 1999b). A metal catalyst can be added to further improve the hydroxyl ion formation (Ong 2006).

Metals are used to improve disinfection mechanisms in a few cases. Pandey and Karanwal (2011) reported that lead and zinc increased the antibacterial effect of ethanolic extract from *Argemone Mexicana* (Pandey, Karanwal 2011). Silvestry-Rodriguez, Sicairos-Ruelas et al. (2007) reported that a synergistic effect is observed when silver is combined with UV light or with an oxidising agent. The presence of other antimicrobials is believed to make the cytoplasm more vulnerable to silver ions which quicken the disinfection (Silvestry-Rodriguez, Sicairos-Ruelas et al. 2007). Kiuru, Sievänen et al. (2011) reported that the combination of an electrochemically produced halogen and sodium percarbonate showed a higher efficiency and lower corrosion than the halogen alone (Kiuru, Sievänen et al. 2011). The use of metal-halogen salts, such as aluminium chloride, silver bromide, and silver chloride, are successfully being used as disinfectant coatings (Leopold, Freese 2009, Sambhy, MacBride et al. 2006). A few sources mention the combination of metallic ions and an oxidising agent as a promising alternative to chlorine treatment (Fewtrell 2014, Martínez, Gallegos et al. 2004).

2.4.6.2 Metal ions and oxidising agent combinations

The combination of metal ions with an oxidising agent is a process combination that was researched in the 1990's, but not in too much depth (Landeen, Yahya et al. 1989, Yahya, Landeen et al. 1990, Pedahzur, Lev et al. 1995, Fewtrell 2014, Cassells, Yahya et al. 1995). Lately, it seems little additional research has been done, but it has been implemented more often (Carefree Clearwater 2015, Aquaking SA 2016, Fewtrell 2014). Research that mentioned such combinations, proposed it as an alternative to chlorine treatment with a direct decrease in chlorine used for disinfection (Pedahzur, Lev et al. 1995, Pyle, Broadaway et al. 1992, Yahya, Landeen et al. 1990). A decrease in chlorine usage will decrease the dangers of chlorine treatment, but probably also some of the advantages. The addition of the metal ions should fill the vacancy of the removed chlorine. Such a combination should be effective in deactivating a wider range of pathogens because of the variety of disinfectant mechanisms involved (Sambhy, MacBride et al. 2006). Other advantages could also include financial benefits, lower environmental impact, and a lower risk disinfection procedure (Pedahzur, Lev et al. 1995).

The disinfection mechanisms of a combined metal ion and oxidising agent treatment procedure are complex and has not been studied (Yahya, Landeen et al. 1990). The metal ions could theoretically react with the oxidants and form other complexes that react differently to the normal disinfecting species present. Theoretically HOCl and HOBr both react with the cell membrane and with the interior of cells, metallic ions also react with the exterior and interior of cells, but the interior of a pathogen is more vulnerable than the exterior (OSU 2011). The oxidation species can therefore weaken the cell membrane or the cell wall to allow metal ions through, which makes the cell interior open to disinfectant actions. The same could be happening other way round (Yahya, Landeen et al. 1990, Lin, Vidic et al. 1996). Therefore, a pathogen might have a resistant membrane to HOCl, but not to silver ions, the silver ions then weaken the membrane causing it to allow other substances through, including HOCl, which then react with the cell interior and cause cell destruction. The contact time requirements for combined treatment is not well documented, but should be thought-provoking, since metal ions require at least double the contact time that oxidising agents require. Referrals to silver-chlorine for point-of-use treatment make it seem that the metal ions enhance the oxidising agent (Fewtrell 2014).

The combination of metal ions and oxidising agents should have several strengths other than the strengths of the individual processes. Firstly, a reduced amount of the oxidising agent can be used if the process is synergistic (Yahya, Landeen et al. 1990). The process should have a low toxicity because silver and copper have a low toxicity and less toxic oxidising agents need to be used. The process should have a long-lasting disinfecting residual, because metal disinfection is known to have a long

residual and some oxidising agents as well (Pedahzur, Lev et al. 1995). A larger variety of pathogens should be susceptible to disinfection because of the different disinfecting mechanisms of the metal ions and oxidising agents (Landeen, Yahya et al. 1989). A final advantage would be a small number of disinfecting by-products, since fewer oxidising agents are used (Pedahzur, Lev et al. 1995).

Metal ions and oxidising agents function on different disinfecting mechanisms, which is a strength, but could be a weakness if these disinfectants react with each other. If the disinfectants react with each other the total efficiency will drop. This should not be the case, however, since metals are oxidised to ionic form at low pH levels and in the presence of oxidising agents, additionally it is the ionic form that becomes the efficient biocide (Dowling, Betts et al. 2003). The precipitation of metal oxides and other uncontrollable reactions would be some of the dangers. The oxidising agent and metal ions are often implemented in series, one after the other, which give some contact time for the disinfectants to react with contaminants before reacting with each other. Controlling pH and maintaining it could also proof challenging with the complex reactions taking place.

There have been different studies on metal ions with oxidising agents, but the results are not comparable, since different pathogens were investigated. Pedahzur, Lev et al. (1995) combined silver treatment with hydrogen peroxide in a ratio of 1:1000 (w). They found that the combined process showed more efficient disinfection than the individual processes, with cases of a synergistic effect. The inactivation rate was slow and the disinfection action seemed similar to chloramines, therefore, it showed promise as a secondary disinfectant with a long-lasting residual for biofilm control (Pedahzur, Lev et al. 1995). Pyle, Broadway et al. (1992) investigated the use of metallic ions with iodine as disinfectant. They combined 100 ppb Cu and 11 ppb Ag and 200 ppb iodine and found these low concentrations efficient for disinfection. The combined treatment was more effective against *Pseudomonas cepacian* than any of the treatments separately. The combined treatment also prevented regrowth, which did occur when only iodine was used (Pyle, Broadaway et al. 1992).

Yahya, Landeen et al. (1990) combined 400 ppb copper, 40 ppb silver and 300 ppb free chlorine and found the combined treatment had a synergistic log reduction when compared to the individual treatment processes. They recommended that copper-silver ionisation should always be implemented with a small amount of free chlorine in swimming pool treatment (Yahya, Landeen et al. 1990). Yahya, Landeen et al. (1989) also showed that the difference in bacterial inactivation between chlorine treatment and copper-silver-chlorine treatment increased with larger chlorine concentrations, Figure 10 (Landeen, Yahya et al. 1989). Martínez, Gallegos et al. (2004) investigated 0.2 ppm silver, 1.2 ppm copper and 0.3 ppm chlorine, and found it controlled bacteria concentrations sufficiently to limit biofilm growth and corrosion due to bacterial action (Martínez, Gallegos et al. 2004). In South Africa,

Aquaking SA (Pty.) has patented technology that combine copper, silver, and zinc ions with the halogen releasing BCDMH (Aquaking SA 2016).

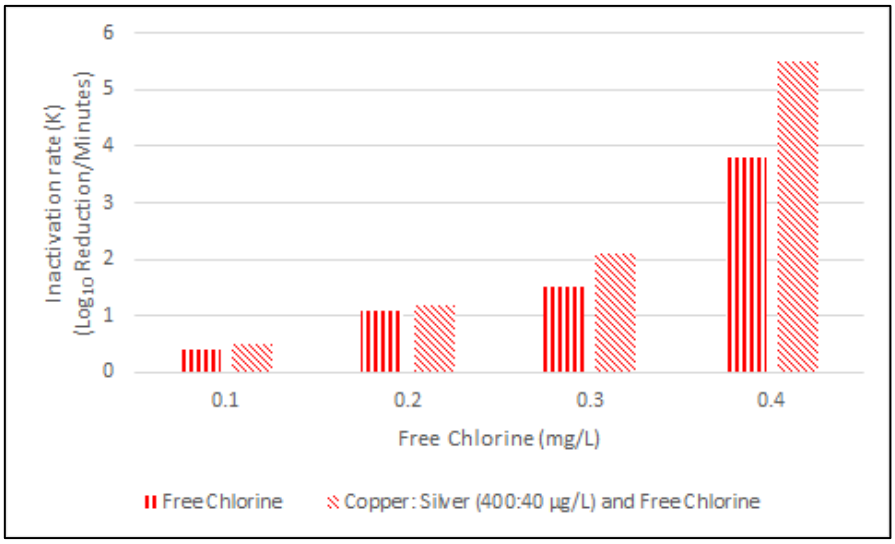

Figure 10: Inactivation rates of chlorine treatment compared to copper-silver-chorine treatment redrawn from Landeen, Yahya et al. (1989)

Abad, Pinto et al. (1994) investigated viral inactivation through a copper-silver-chlorine treatment. They combined 700 µg/L copper and 70 µg/L silver with 0.5 or 0.2 mg free chlorine, and found that viral inactivation was comparable to inactivation found with higher free chlorine dosages, but the addition of metal ions did not enhance the viral inactivation rates. The research did not promote copper-silver ionisation as an alternative to chlorine treatment, although it did mention the residual presence of copper and silver (Abad, Pinto et al. 1994).

2.5 Assessment and control of water disinfection

2.5.1 An overview

Water is a natural resource that is essential for most of our daily activities. The identification of water contaminants led to the need to control water quality and ensure water standards (Schutte, Focke 2006, Hall, Van Koppen et al. 2014). Water pathogens in drinking water often lead to sever sicknesses and even death. Adequate disinfection of drinking water sources is, therefore, essential (Fewtrell, Bartram 2001). Water utilities treat large volumes of water before distributing it in the water network where thousands of people are exposed to it. Inadequate disinfection will lead to large disease outbreaks, examples include the cholera outbreaks often seen in developing countries (Kouadio, Aljunid et al. 2012, Nash 1992, Griffith, Kelly-Hope et al. 2006). There is therefore a need to quickly assess the effectiveness of disinfection to control disinfection and ensure effective disinfection.

The main aim of assessing and controlling disinfection is to ensure water quality without using too much disinfectants. Human health is of utmost importance and therefore water utilities need a degree of certainty that water disinfection is successful in removing most pathogens (World Health Organization 2008). The disinfection assessment should, therefore, always be able to identify when disinfection is inadequate and inform operators. On the other hand, excessive treatment is expensive and can also have adverse health effects on water users. Excessive use of chemicals during treatment can cause the formation of dangerous by-products or can affect humans directly when exposed to skin or orally consumed (Hrudey 2009, Pressman, Richardson et al. 2010).

Considering the objective of disinfection assessment and control, several ideal characteristics can be identified that would make such a control procedure very efficient. The first characteristic would be to give continuous feedback on the presence of any microbiological contaminates in the water. Secondly, the assessment should give continuous feedback on the amount of disinfectant present. If these assessments can be automated and continuous, they can be connected to the treatment process that can ensure exact disinfectant release triggered by the levels of contamination. Water quality will then be of a constant quality and treatment costs will be minimised.

The different approaches currently used to assess microbiological water quality and disinfection success can be summarised as microbiological and industrial methods. Several microbiological approaches exist that focus on determining the actual pathogen concentrations and the different species that are present. These approaches are discussed in *2.5.2 Microbiological methods* and differ vastly, as some count single organisms and others make use of other characteristics that indicate pathogen presence. Microbiological approaches focus on the presence or absence of the pathogens. Industrial approaches to assess disinfection usually focuses on the disinfectant applied and changes in physical or chemical characteristics of the water that is treated. These approaches are discussed in *2.5.3 Industrial methods* (World Health Organization 2008).

2.5.2 Microbiological methods

There are various methods for determining bacterial concentrations and bacterial presence. These methods form part of the microbiological methods that are used to assess and control disinfection. This section discusses some of these methods implemented, how they work, their advantages and their disadvantages. Plating and optical density (OD) are discussed in detail, while gas release monitoring, turbidity and Colilert are only discussed shortly. These methods generally make use of the pathogen indicator principal discussed in *2.3.2.2 Pathogens*.

Diluting, plating and plate counting form part of the common microbiological process used to determine bacterial concentrations. Sterile petri-dishes are prepared with a specific growth-medium, to allow for specific bacteria cultures to grow. The solution, to be investigated, is diluted in increments of a tenth in saline solution (9 g NaCl/L) and then plated on the growth-media in the petri-dishes. The different growth media will allow different bacteria cultures to grow. After sufficient incubation time the bacterial colonies can be counted on the petri-dishes and an estimated bacterial concentration can be calculated in colony forming units (cfu) per millilitre. The calculated bacterial concentration is still only an estimation, and not an exact indication of bacterial concentration. The process is time consuming and special sterile working conditions are needed to validate the results (Huang, Shih et al. 2008, Fisher, Thornton et al. 1922, Jennison, Wadsworth 1940). Concentrations of heterotrophic colonies, *E. coli* and other indicator bacteria are often measured through plate counting (Kusić, Kampe et al. 2014, Tanchou 2014, Huang, Shih et al. 2008, Fisher, Thornton et al. 1922, Jennison, Wadsworth 1940).

Optical density (OD) is often used as a quick microbiological method to determine bacterial concentrations. OD is measured in a spectrophotometer by measuring the amount of light scattered by a sample in a cuvette. The OD is measured for a specific wavelength and compared to a cuvette with a sample without any bacterial content. The principle is that the bacterial cells cause the light rays to scatter and increase the OD. As bacteria concentrations increase it allows less light through the media, because of the physical presence of living and dead bacterial matter, as well as substances secreted by the bacteria. Measured OD and bacterial concentrations are usually directly proportional to each other for the exponential growth phase of a bacterial culture. OD is often used when monitoring a bacterial culture's growth. Bacterial concentrations cannot be determined from OD for complex cultures with a variety of constituents and where the bacteria culture is no longer in the exponential growth phase (Widdel 2007).

The first microbiological assessments for bacterial presence measured the amount of gas produced in sealed containers (Symons 2006). There are several tests that measure a change in gas concentration or gas release, these gases include dissolved oxygen (DO), carbon dioxide, and hydrogen sulphide (H_2S). A decrease in DO and a detectable release of carbon dioxide both indicates aerobic bacteria present in water. Hydrogen sulphide tests are often referred to as the paper strip test method, and measure the presence of H_2S which serves as an indication of certain types of bacteria present (World Health Organization 2002, Clarke 1953). New developing technology include biological mass spectrometry (BMS). BMS is promising new technology for rapid characterisation of micro-organisms (Zhaoguang, Zhongxian et al. 2008). The Colilert has become the easiest and quickest method to

determine total coliform and *E. coli* concentrations, although it becomes expensive to use repeatedly (Covert, Shadix et al. 1989, Cowburn, Goodall et al. 1994, Fricker, Illingworth et al. 1997).

2.5.3 Industrial methods

Disinfection is often implemented in large industrial application where the microbiological methods of assessing disinfection cannot be practically employed. Industrial methods have therefore been developed that can quickly and efficiently be used to get an indication of disinfection success. Usually, Industrial methods are focused on the disinfectant applied, its residual or its intensity. Statistical analysis of years of treatment data has correlated the relationship between amount of disinfectant required and disinfection success.

As mentioned before, chlorine is the most commonly used disinfectant and therefore its dose control is of significance. Free chlorine and total chlorine does not correlate with disinfection on their own, primarily due to the speciation at different pH (Thomas 2006, Kim, Hensley 1997). Therefore, chlorine is monitored by measuring the free chlorine concentration in combination with pH, which gives an indication of chlorine concentration as well as the active chlorine species (Devkota, Williams et al. 2000). The pH can be adjusted by adding hydrochloric acid to decrease pH or sodium carbonate to increase pH, alternatively, chlorine can be added to adjust a chlorine deficiency (Bastian, Brondum 2009). A free chlorine residual is usually maintained at a certain pH to ensure primary and secondary disinfection (Haas, Joffe et al. 1996).

The main drawbacks of chlorine residual measurements are the complexity of chlorine break-point behaviour, complexity of chlorine speciation and the dependence on pH of chlorine's disinfecting ability (Devkota, Williams et al. 2000). Chlorine demand is not consistent, with spikes depending on water quality. Periodic measurements of chlorine residual can, therefore, lead to insufficient treatment (Thomas 2006, SABS 2015b). Free chlorine is measured using DPD colorimetric methods or amperometic titrations, usually periodically, although technology is being developed that can monitor it continuously (Hall, Zaffiro et al. 2007, Kim, Hensley 1997). Free chlorine does not differentiate between hypochlorous acid, which is a stronger biocide, and hypochlorite anions, and does not take chloramines into account. Total chlorine does not differentiate between inorganic chloramines, which is a good biocide, and organic chloramine, that has no biocidal effects (Kim, Hensley 1997, Devkota, Williams et al. 2000).

Turbidity can be described as the industrial version of optical density and is measured in NTU. Turbidity, however, does not measure only bacterial contaminants, but includes all other particles in the water. Turbidity is an effective quick indication of water quality (Schutte, Focke 2006, World Health

Organization 2008). Other water quality measurements often monitored in industries are electric conductivity (EC), total dissolved solids (TDS), total oxygen demand (TOD), chemical oxygen demand (COD), dissolved oxygen (OD), and oxidation reduction potential (ORP) (Schutte, Focke 2006, Hall, Zaffiro et al. 2007, World Health Organization 2008). These measurements can be monitored real- or near-real time which improves response time. The monitoring of ORP and DO has been given extra attention because so many disinfectants are oxidising agents (Ndegwa, Wang et al. 2007). A quantitative and qualitative real-time monitoring process would be ideal to control disinfection processes. A dual oxidation control system (DOCS) has been designed to combine ORP, pH and free chlorine monitoring and build on the individual monitoring strengths (Thomas 2006).

2.5.4 Oxidation reduction potential (ORP)

Oxidation-reduction reactions are often referred to as redox reactions, these reactions refer to the transfer of electrons between atoms, molecules, or ions (James, Copeland et al. 2004). Oxidation refers to the "loss" of electrons that is experienced by the reductant, and reduction refers to the "gain" of electrons experienced by the oxidant (Singer, Reckhow 1999). The oxidation-reduction potential (ORP) measurement is an indication of the free electrons available in the solution and the oxidising or reducing tendency of the solution (Sigg 2000). The ORP is dependent on the concentrations of all the chemicals in the solution except for water (H_2O) itself (Copeland, Lytle 2014, Rosemount Analytical Inc. 2008). Schmelkes (1933) was the first to identify ORP as a possible process control for chlorination in 1933 (Schmelkes 1933). ORP has since been investigated as a surrogate qualitative control in a variety of water treatment processes, but has not been implemented widely (James, Copeland et al. 2004, Copeland, Lytle 2014, Koch, Oldham 1985, Lund 1963, Bastian, Brondum 2009, Thomas 2006, Devkota, Williams et al. 2000, Schmelkes 1933).

ORP is measured with an ORP probe, which is a millivolt (mV) meter that measures the potential across two electrodes (Hybrid Turkeys 2013, Devkota, Williams et al. 2000). ORP probes consist of two electrodes, an inert electrode, often platinum that is in contact with the solution, and a reference electrode, often silver in a silver chloride solution (Spencer, Aquametrix 2013). The potential difference between the measured solution and the reference electrode is the ORP of the solution (Rosemount Analytical Inc. 2008, Ndegwa, Wang et al. 2007). The ORP can also be calculated theoretically using the Nernst equation (Equation 17) (Sawyer, McCarty 1967). Any oxidising agents will therefore increase the ORP and reducing agents will decrease the ORP which makes ORP a reflection of the redox state of the solution (Dabkowski 2008, James, Copeland et al. 2004, Copeland, Lytle 2014).

$$E = E° - \frac{RT}{zF} ln \frac{[reductants]}{[oxidants]} \quad \text{(Eq. 17)}$$

Where:

- E = ORP of the solution, volts;
- E° = ORP of the solution in the standard state, volts;
- R = universal gas constant, 8.314 J/mol•K;
- T = absolute temperature, K;
- z = number of equivalents per mole (2 in this case);
- F = Faraday's constant, 96 500 Coulomb/mol; and
- [] = concentration, mol/L (Sawyer, McCarty 1967).

ORP is sometimes used as a control for monitoring oxidation-reduction reactions and determining the state of reactions (Rosemount Analytical Inc. 2008). When measuring ORP values for different oxidising agents, strong relationships have been established under controlled conditions. It has been proven that there is a strong linear correlation between ORP and the log of dissolved oxygen (Ndegwa, Wang et al. 2007). Figure 11 shows the different concentrations of free chlorine that correspond with different ORP values at specific pH. These, however, are pure experimental situations where the conditions are well controlled, and not as applicable to raw water. ORP is not effectively used to measure concentrations of ions because it is affected by all the ions and the state of the half reactions occurring in the solution (Kim, Hensley 1997). In depth understanding of the half-reactions taking place, the pH, and the state of the half-reaction equilibrium as well as the effect that temperature has on the half-reactions, is needed to be able to calculate ion concentrations from ORP readings (Rosemount Analytical Inc. 2008).

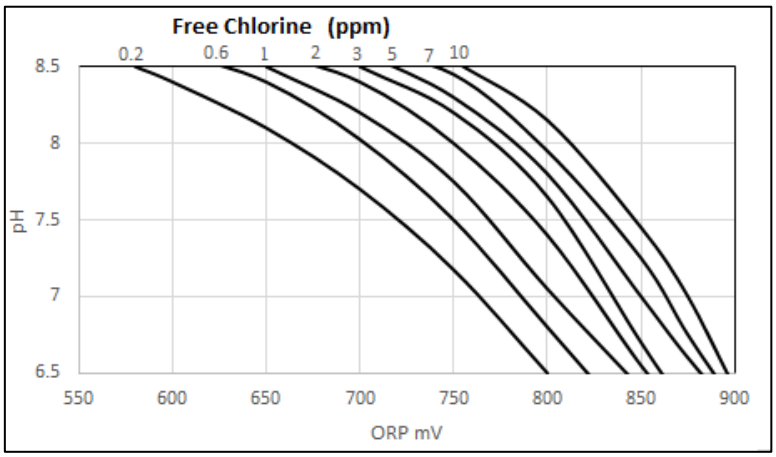

Figure 11: ORP values of different chlorine concentrations at different pH redrawn from Steininger, Pareja et al. (1996)

Since ORP reflects the redox state of a solution, it can be used to monitor oxidising disinfectants. Oxidising disinfectants with better disinfecting capabilities are usually stronger oxidising agents and therefore have higher ORP values for specific concentrations (Thomas 2006). Biocidal efficiency shows a stronger correlation to ORP readings than either pH or fee chlorine residual on their own (Victorin, Hellström et al. 1972, James, Copeland et al. 2004). Higher ORP values require lower contact times as can be seen by the experimental data of Suslow in Table 3 (Thomas 2006, Suslow 2004). Research investigating disinfection has shown that an ORP of 650 mV destroys bacterial and viral pathogens within seconds regardless of the oxidant used (Lund 1963, Bastian, Brondum 2009). Only pathogens known to be resistant against the different disinfectants continued to show resistance above an ORP of 650 mV (Suslow 2004). ORP is a qualitative indication of water quality and not a quantitative indication that gives a window of operation rather than a point of operation (Suslow 2004, Thomas 2006).

Table 3: Pathogen survival at different ORP adapted from Suslow (2004)

	Survival (seconds)		
ORP Value (mV)	< 485	550 to 620	665
E. Coli 0157:H7	> 300	< 60	< 10
Salmonella spp.	> 300	> 300	< 20
Listeria	> 300	> 300	< 30
Thermolent Coliform	> 48 hours	> 48 hours	< 30

Lund's Law, Equation 18, is a model to project the rate of bacterial disinfection for different ORP values compared to the threshold ORP, which is the minimum ORP required to kill bacteria (Devkota, Williams et al. 2000, Lund 1963). This model has potential, but the determination of the threshold ORP has its own challenges.

$$ln\frac{N}{N_0} = -k'(E - E_c)^n t \qquad \text{(Eq. 18)}$$

- N_0 = initial coliform population, MPN/100 mL;
- N = coliform population at time t, MPN/100 mL;
- k' = coliform inactivation rate constant;
- E = ORP level maintained at contact time t, mV;
- E_c = Lower threshold ORP below which no kill occurs, mV;
- t = contact time, minutes.

Chlorine disinfection can theoretically be controlled by ORP. Chlorine treatment causes a variety of chlorine species to form depending on the water conditions which complicate the monitoring and control of chlorination. At low pH, free chlorine forms hypochlorous acid (HOCl) and at higher pH, hypochlorite ions (OCl^-). In the presence of ammonia chlorine can form chloramines that also have biocidal properties, but not as strong as HOCl. When monitoring chlorination, an increase in pH decrease ORP readings, and a decrease in free chlorine decrease ORP readings (Bastian, Brondum 2009). Similarly, an increase in chlorine demand causes a drop in free chlorine and ORP levels (Hall, Zaffiro et al. 2007). The ORP of different chlorine species at different concentrations can be seen in Figure 12, which shows the stronger chlorine species with stronger biocidal properties have larger ORP values. Therefore, the ORP of chlorine treatment should be directly related to the biocidal ability of the available chlorine. However, ORP has not replaced free chlorine monitoring due the complexity of the reactions that take place (World Health Organization 2008, Steininger, Pareja et al. 1996).

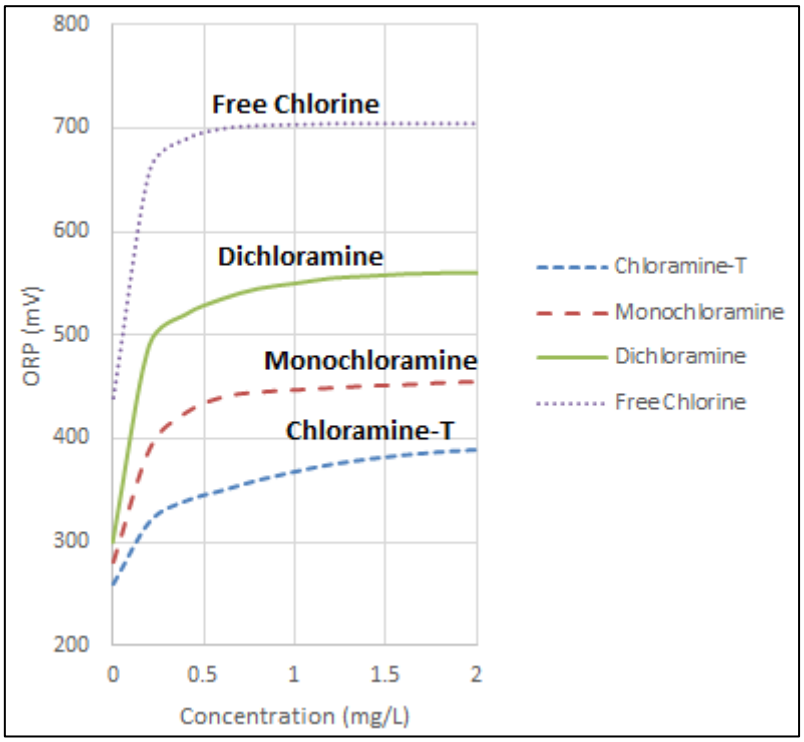

Figure 12: ORP of different chlorine species redrawn from Victorin, Hellström et al. (1972)

ORP enables the effectiveness of water disinfection to be measured, when oxidation is used as disinfectant, regardless of the source of disinfection (Hybrid Turkeys 2013, Bastian, Brondum 2009, Steininger, Pareja et al. 1996). ORP gives an in-line, real-time quality indication that can be combined with an automated treatment system that optimises operation and ensures more control (Koch, Oldham 1985, Bastian, Brondum 2009, Steininger, Pareja et al. 1996). Changes in the chemical composition of water can be picked up immediately and chemicals can be activated on demand (Kelley

2004, Thomas 2006). A wide variety of processes can be monitored and controlled including maintaining a chlorine residual, ensuring dichlorination and monitoring nutrient removal (Dabkowski 2008, Kelley 2004, Kim, Hensley 1997). Overall, ORP monitoring is simpler and cheaper than chlorine residual monitoring and improves safety, reliability, and effectiveness of the disinfecting procedure (Dabkowski 2008, Kelley 2004, Suslow 2004).

ORP is not implemented as commonly as would be expected, generally due to the complexity of the oxidising agents in water (Bastian, Brondum 2009). Firstly, ORP is not quantitative and cannot be used to calculate actual chemical concentrations because the ORP values of raw water fluctuate so drastically (Bergendahl, Stevens 2005, Devkota, Williams et al. 2000). ORP values are not absolute, but relative to the solution's background ORP, which is affected by all the elements in the solution (Devkota, Williams et al. 2000). To reach targeted 650 mV ORP values for disinfection, over treatment may occur that can cause other dangers (Bastian, Brondum 2009). ORP probes can be problematic, as sensors become fouled, a 10 minute waiting period is required to get accurate ORP readings, and duplicate ORP readings often show inconsistency (James, Copeland et al. 2004, Copeland, Lytle 2014, Steininger, Pareja et al. 1996, Suslow 2004). Further equipment problems include maintenance, calibration, cross-checking, and oxide and sulphide coatings on probes, while temporary saturation sometimes cause a lag response for probes (Ndegwa, Wang et al. 2007, Suslow 2004).

The implementation of ORP technology has grown steadily over the past few decades and suggest many potential applications even though there is limited data available on ORP use (World Health Organization 2008, Bastian, Brondum 2009). The WHO recommends that single ORP values that ensure disinfection should be determined case-by-case and not defined universally (World Health Organization 2008). As disinfectant control ORP is still not often implemented on its own, because it cannot prevent over- or under treatment. The use of a dual oxidation control system (DOCS) that matches oxidant feed to ammonia and organic loads, is gaining popularity (Thomas 2006). ORP has also been found to display features in correlation with wastewater stabilisation (Ndegwa, Wang et al. 2007). Aeration processes as well as different respiratory activities in bio-reactors are effectively monitored by ORP (Koch, Oldham 1985, Ndegwa, Wang et al. 2007). ORP monitoring can further be implemented in biological nutrient removal systems with nitrogen removal and phosphorus release (Charpentier, Godart et al. 1989, Dabkowski 2008, Koch, Oldham 1985).

2.6 Summary of literature review

Water quality has deteriorated to a state that water treatment and water disinfection has become necessary to provide potable water. Chlorine disinfection has been, and still is, the most commonly

used disinfectant. Chlorine has several disadvantages that has supported the development of alternative disinfecting technologies. UV, ozone and copper-silver ionisation are some of the disinfecting treatments that are gaining popularity. The combination of metal ions with an oxidising agent is one of the alternative technologies that have appeared on the market. There is growing evidence that the combined technology is more efficient than simply the addition of the separate processes. The disinfecting mechanisms of metal ions and oxidising agents differ and the combined interaction on pathogens is not yet understood.

Disinfection efficacy can be assessed by measuring the actual pathogen concentrations or by looking at excessive disinfectant presence. Chlorine disinfection is usually monitored by the free chlorine residual and the corresponding pH. Oxidising disinfection processes are seldom monitored continuously, which creates the possibility for lapses or spikes in treatment. Oxidation reduction potential (ORP) has been recognised as a qualitative indicator for water disinfection treated with an oxidising agent. The oxidising strength of a chemical often correlates strongly with its biocidal efficiency. Some literature sources say pathogens are destroyed within seconds at an ORP above 650 mV, while other sources mention the change in ORP and a relations to disinfection success. ORP is, however, not a quantitative measure and is influenced by most of the elements in the water.

3.Experimental methodology

This chapter serves to lay the foundation and understanding for the experimental process. Section *3.1 Introduction* is a broad explanation of the different experimental components and a summary of the final experimental process. The sections that follow explain how the experimental process was determined and the challenges that had to be overcome. Section *3.2 Feed development* explains what the final treatment feed was and how it was chosen. *3.3 BCDMH treatment* discusses the choice of oxidising agent and method of treatment. *3.4 Metal ion treatment* is an extensive investigation into metal release through ionisation, controlling it, and measuring it. Section *3.5 Assessment and control of disinfection* discusses the choice of assessment for disinfection and other controls put in place. 3.6 Apparatus is the final experimental equipment, apparatus and procedures. The last section, *3.7 Analysis of experimentation*, explains the statistical analysis tools used to investigate experimental data and create models.

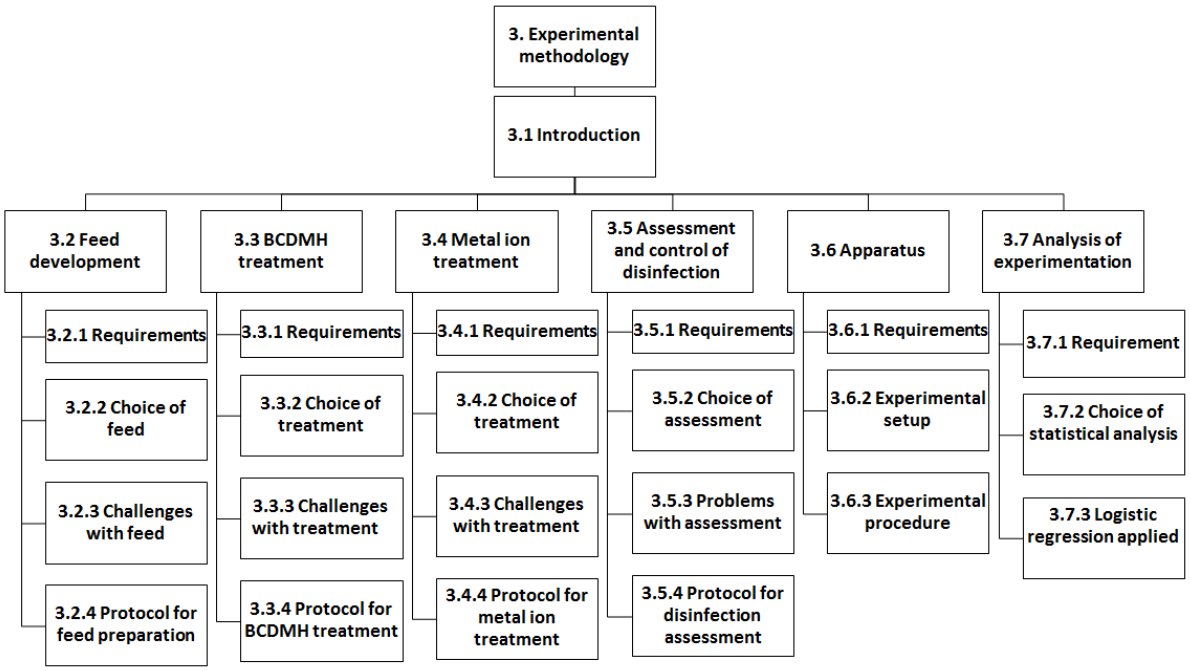

Figure 13: Experimental methodology structure

3.1 Introduction

The main objective of the experimental methodology is to have an experimental process that can be used to generate the required data to achieve the objectives of the thesis. An experimental setup and procedure was developed to investigate the efficiency of a combined metallic ion and oxidising agent treatment process. Figure 14 shows the main components of such a system. Firstly, the feed had to be established that was to be used for experimentation. Secondly, the method of treatment for the oxidising agent, BCDMH, and the metal ions had to be determined. Thirdly, the assessment and control

of disinfection had to be developed. The components of the experimental process were then combined in the apparatus. Finally, an appropriate set of statistical analysis tools were used to analyse the experimental data.

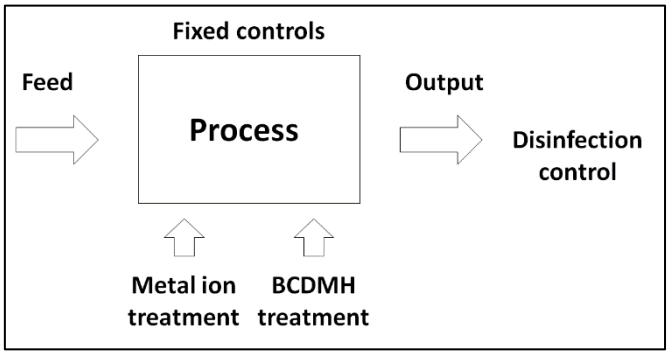

Figure 14: Experimental overview

The final experimental methodology can be summarised as follows. The feed consisted of tap water was artificially contaminated with *Pseudomonas sp. strain CT07* at a concentration of between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/mL. Metal ion treatment consisted of the ionisation of copper, silver and zinc and was measured by the coulomb electrons released through ionisation. BCDMH treatment was done using a BCDMH stock solution and measured in concentration treatment. Bacterial plate counts were used to determine disinfection and ORP was monitored as an additional disinfection control. The experimental setup consisted of batch experiments that treated 900 mL for a constant contact time of 5 minutes with a combination of metal ions and BCDMH. Logistic regression was used to analyse the results and determine the interaction between the disinfectants.

3.2 Feed development

3.2.1 Requirements of feed

The feed was an important part of the experimental process. As discussed in the literature in *2.4.3 Factors that influence disinfection*, the physical, chemical, and microbiological properties of water influence disinfection success. Therefore, the consistency of the feed had a direct effect on treatment success and was developed in such a way that it had a minimal influence on disinfection. Additionally, the feed had to represent a contaminated water source that could be used to compare to disinfection. Therefore several requirements were determined to which the developed feed needed to comply with.

The feed needed to represent a contaminated water that would be relevant to point-of-use water treatment. The feed had to be quantifiable after treatment as either successful or unsuccessful. The

physical properties, i.e. pH, temperature, turbidity, and electric conductivity (EC) had to be relatively constant as not to interfere with disinfection. The chemical properties, i.e. salinity, alkalinity, hardness, and organic content was kept reasonably constant as not to influence disinfection. The microbiological properties, i.e. the pathogens present and their concentration had to be constant to be able to have repeatable measurements of disinfection.

The feed needed to be of a maximum biosafety level 1 to ensure the safety of the student and to ensure experiments were to be conductible in the laboratory. The feed had to be contaminated by a bacterium that responded similarly to well-known pathogens such as *E. coli*. The bacterial concentration of the feed had to be easily determined, measured and had to ensure repeatability of experiments.

3.2.2 Choice of feed

To test the efficiency of disinfection technology, several approaches can be implemented. The first choice is between experimenting on a real water source that is already contaminated, or to artificially contaminate a water source. When an artificially contaminated feed is chosen, the feed can be contaminated by multiple organisms or by a singular pure culture. When investigating a singular organism, a dangerous relevant pathogen can be investigated or a non-pathogenic organism that could serve as a pathogen indicator. These different choices all have their limitations, but considering the feed requirements, the only plausible options were to develop a single culture feed that would be artificially contaminated by either the known pathogenic *E. coli* or an alternative less pathogenic bacteria culture.

Pseudomonas sp. strain CT07 was chosen as the single pure bacterial culture to be used as contaminant in the feed. *CT07* was chosen as contaminant, because it is a waterborne bacterium that has similar characteristics to *E. coli*, but is safer and easier to work with since it is a biosafety level 1 pathogen. Several other studies have made use of bacteria from the *Pseudomonas* family to investigate disinfection (Wirtanen, Salo et al. 2001, Yahya, Landeen et al. 1990). It was decided to use sterilised tap water as water medium for the feed. The physical and chemical characteristics of tap water might vary slightly, but it reflects a practical and realistic water source with a natural amount of nutrients, salts, and minerals. The bacterial concentration chosen was to be comparable to a highly contaminated river. Excessive bacterial concentrations were preferred above low bacterial concentrations to ensure the disinfection challenge.

From here on *CT07* will be used to refer to the *Pseudomonas sp. strain CT07*. *CT07* have the added advantage of forming biofilm when exposed to environments for prolonged time, which expands the

experimental potential for investigating planktonic and sessile reactions to disinfectants. The *CT07* stock was streaked from liquid media that was inoculated, grown, and inoculated again from a freezer culture. The *CT07* strain was isolated from a sample taken from a cooling tower at Stellenbosch University, South Africa (Bester, Wolfaardt et al. 2005).

3.2.3 Challenges with feed

The *CT07* feed had several challenges that had to be understood and overcome before the feed could be used for experimentation. The main question was how to grow *CT07* repeatedly to the same concentration and to then be sure the concentrations were relatively constant. A general understanding of the growth patterns of *CT07* was therefore necessary. Microbiological plating and optical density (OD) had to be investigated to determine quick methods of determining *CT07* concentrations. Growth curves were repeated to ensure the repeatability of feed preparation. Finally, a number of controls were put in place that limited variability in *CT07* concentrations.

To determine the general growth pattern of *CT07*, a full growth curve was done over 24 hours. Three bacteria cultures were grown from different stock cultures and full dilutions and plating were done every 2 hours to create the full 24-hour growth curve with standard deviations. The plates were left to grow for 48 hours at room temperature. The average bacterial concentrations can be seen for the three cultures grown on Figure 15, the standard deviation is so small that it cannot be seen.

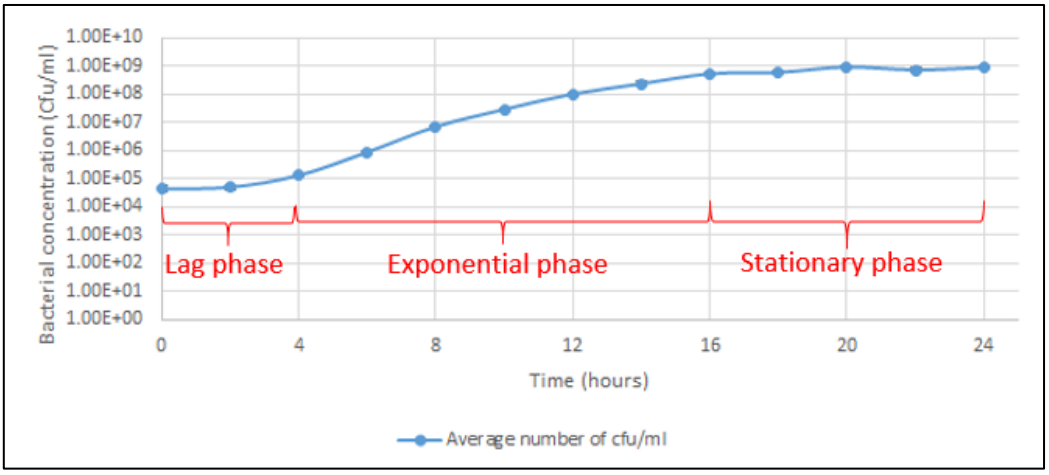

Figure 15: 24-hour growth curve for CT07

The lag phase is the first 4 hours, the exponential growth phase is the next 12 hours and the stationary phase is after 16 hours. The stationary phase was identified as the phase to be used for experiments, because it ensures repeatability as bacterial concentrations are then relatively constant. The stationary phase also ensures the feed is usable for a few hours as it remain constant, therefore it simplifies experimental control.

As an alternative method to determine bacterial concentrations, absorbance was investigated and compared to bacterial concentrations calculated through plating. A wavelength of 600 nm was used for the absorbance, i.e. OD_{600}, and the absorbance was measured every 2 hours for 24 hours with three samples just as the bacterial concentration growth curve was done. The OD_{600} versus time curve can be seen for the full 24-hour growth on Figure 16.

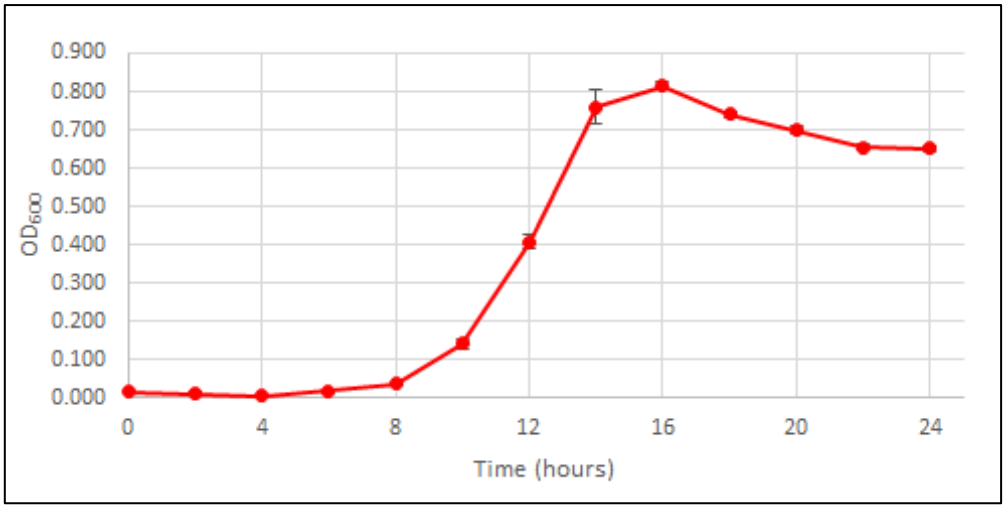

Figure 16: OD_{600} for 24-hour growth curve

The growth phases are recognisable, with a lag phase the first four hours, exponential growth the next ten hours and a decrease in OD_{600} observed after 16 hours. A difference between the bacterial concentration and OD_{600} curves can best be seen when they are plotted against each other, Figure 17.

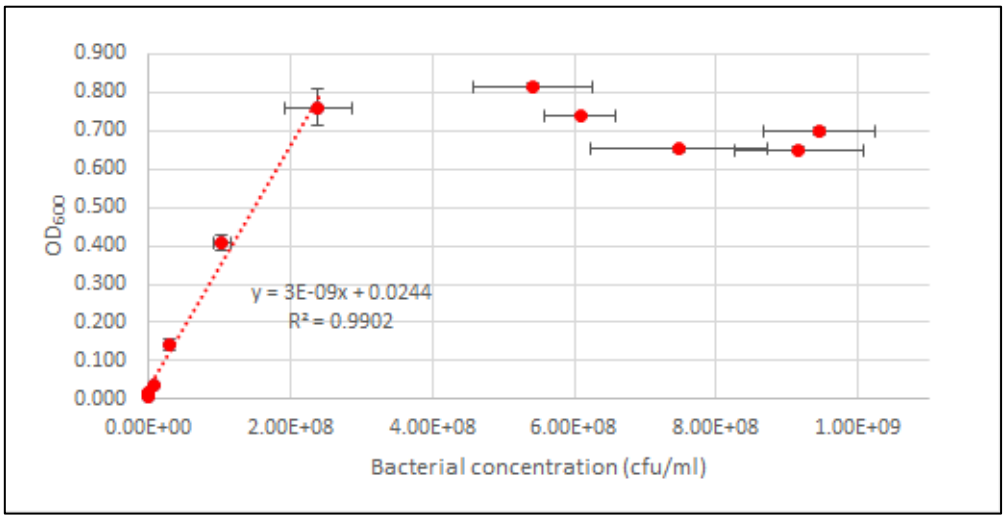

Figure 17: OD_{600} vs bacterial concentration

The OD_{600} relationship is linear compared to the bacterial concentration for the first 14 hours, with an R-squared value of 0.9902, but thereafter there is no linear relationship. The OD_{600} increases until it reaches a maximum value after which it starts to decrease slowly even when the bacterial

concentration is increasing. The OD_{600} will therefore not be a sufficient measurement on its own to determine an estimated bacterial concentration during the stationary phase.

The variability of bacterial growth is not noticeable on the full growth curve with the log axis, Figure 15. The three cultures were grown at the same time from different stock cultures, but showed repeatable results. The repeatability had to be investigated for different days. Three different cultures were grown on three different days, but only the growth curve for the stationary phase was investigated, i.e. bacterial concentrations and OD_{600} was measured for 16-hours, 18-hours and 20-hours. Figure 18 represents all the data of the six cultures grown for the stationary phase, 3 cultures grown on the same day and 3 cultures grown on separate days. 16-hours seem to be the start of the stationary phase, with concentrations still increasing to the 18-hour mark. From 18-hours the bacterial concentrations are more than 0.5 $x10^9$ cfu/mL and less than 2.0 $x10^9$ cfu/mL. A bacterial concentration of between 0.5 $x10^9$ cfu/mL and 2.0 $x10^9$ cfu/mL was accepted as a relative repeatable concentration for *CT07* grown for 20 hours.

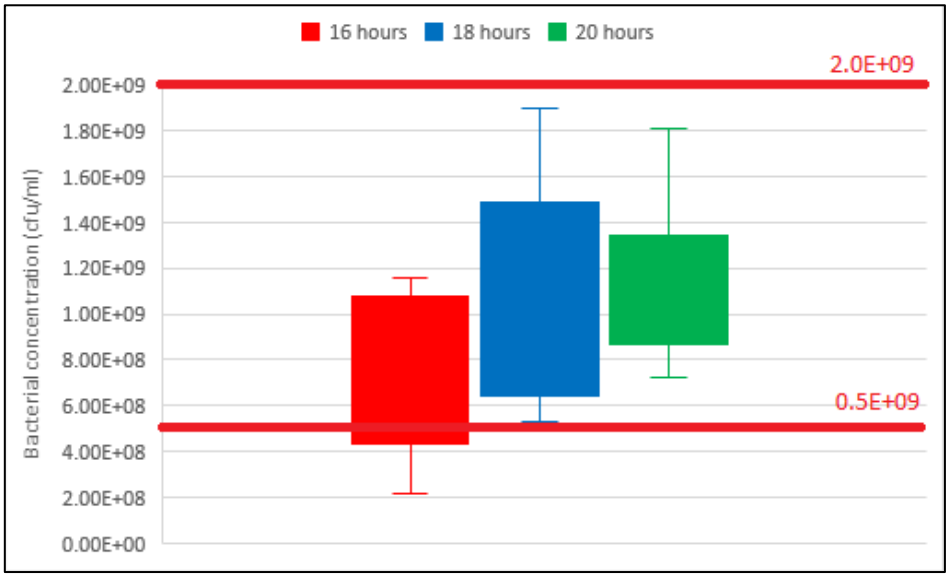

Figure 18: Box and whisker diagram for stationary growth phase for all growth data recorded

Coliform bacterial contamination can vary from 0 cfu/mL to 10^6 cfu/mL, depending on the water source (Ackermann 2010). It is therefore difficult to quantitatively define efficient disinfection. It was decided to create a worst-case scenario for the batch treatment process. The Plankenberg River, outside Stellenbosch, has bacterial concentrations of up to 10^6 cfu/ml, which is high for a natural water source (Ackermann 2010). To test the robustness of the disinfection procedure a bacterial concentration of 10^7 cfu/ml was chosen as a feed concentration. The bacterial cultures grown in TSB were diluted by 1:100 to achieve a concentration between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/ml. The treatment procedure had to decrease bacterial concentrations to less than 10^2 cfu/ml, 10 cfu per 100 µl plated,

to have been determined as successful in disinfection. This means a 5-log reduction was required by the disinfection process to pass the bacterial deactivation requirements.

The physical, chemical, and microbiological characteristics of tap water feed were expected to vary slightly. However, it was assumed that the characteristics would still be within a range that would not influence the disinfection. The slight variability in the feed was chosen to determine the practical application of the treatment on water from natural sources. Tap water was expected to have a low chlorine residual and small amounts of nutrients, minerals, and salts. The chlorine residual and any microbiological content was expected to be removed when the water was sterilised in the autoclave. The nutrients, minerals and salts were of value as they would support the survival of the *CT07* and prevent it from dying due to a lack of nutrients.

A quick bacterial concentration indicator was difficult to develop. The OD_{600} would have worked for low concentrations that fall within the exponential growth phase, but at the stationary phase there was no relationship. Figure 19 shows two OD_{600} graphs compared for 24-hour growth curves on different days.

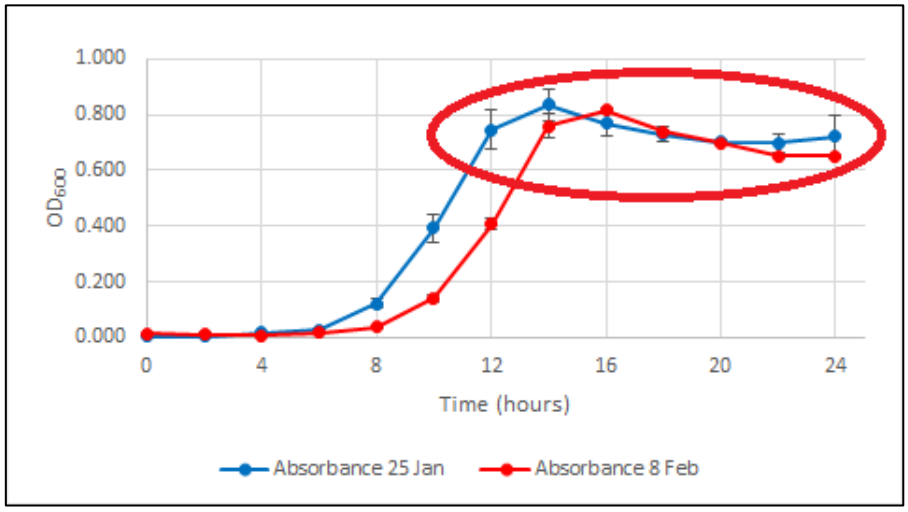

Figure 19: Comparing OD_{600} curves for cultures grown on different days

The curves have the same shape and pattern, while a slight delay for the 8 February curve can be observed. The OD_{600} did not drop to below 0.600 again within the 24-hour period. An OD_{600} above 0.600 can therefore be an indication that the bacterial culture had reached its stationary phase. The growth curves showed repeatability when comparing culture age and concentration and this could function as an indicator of expected bacterial concentrations. The OD value is very quick to get from the spectrometer, and therefore useful in combination with the age of the culture which will give an indication of growth phase and concentration. A bacterial culture that is older than 16 hours with an

OD_{600} of above 0.600 was, most probably, in the stationary phase at a concentration of about 10^9 cfu/ml.

3.2.4 Protocol for feed preparation

The feed preparation was an extensive process that involved steps that needed to be completed a few days before treatment. The initial step was the preparation of the Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) a few days before experimentation. The actual growth of the feed starts a day before experimentation.

The TSB was inoculated with *CT07* 20 hours before treatment. After inoculation it was incubated at 32°C until experimentation. After 20 hours, a 1 mL sample was removed from the bacteria containing TSB and the absorbance was measured. If the OD_{600} was above 0.600 then the bacteria culture is ready to be used for treatment. Another 1 mL sample was removed from the bacteria culture and diluted and plated to determine the actual bacterial concentration. 891 mL sterilised tap water, measured with the measuring cylinder, was added to a 1 000 mL treatment container. 9 mL bacteria containing TSB, was added with a pipette to the sterilised water. The magnetic stirrer was set to 200 rpm. The treatment container contained 900 mL liquid, consisting of tap water with a bacterial concentration between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/ml.

The feed characteristics can be summarised as follows:

- *CT07* at a concentration between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/mL in tap water.
- For the batch experimentation 900 mL feed was used.

To ensure the feed was within bounds the following was maintained:

- Bacterial cultures were grown for 20 hours
- OD_{600} will was measured and should be above 0.600
- Bacterial concentrations were determined through diluting and plating
- If bacterial concentrations were determined to not to lie between the region of 0.5 $x10^9$ and 2.0 $x10^9$ cfu/mL for the feed source, the experiment was declared void.

3.3 BCDMH treatment

3.3.1 Requirements

The broader objective of the research was to improve understanding of ionisation-oxidation disinfection and the possible disinfecting mechanisms involved. Since oxidation disinfecting technologies differ greatly in mechanisms and efficiency, a single oxidising agent had to be chosen

that could be investigated to widen the understanding of ionisation-oxidation disinfection. An oxidising agent that is easy to work with, easy to store, chemically understood, and possibly beneficial over chlorine was ideally needed for the investigation. Taking these characteristics into account, bromo-chloro-dimethyl-hydantoin (BCDMH) was chosen as oxidising agent. The research objectives were determined following this logic for choosing BCDMH as disinfectant. The application of BCDMH treatment had a few requirements.

Firstly, the treatment had to be similar to BCDMH water disinfection implemented in the industry. This was practically difficult as little data was available on actual BCDMH application, and the values varied depending on the scale of contamination. BCDMH treatment is often implemented in a tablet form, where it is usually placed in a flow system to slowly release the halogens and disinfect. This form of treatment has very limited control over the actual BCDMH mass that reacts with water and forms part of treatment, i.e. the BCDMH treatment concentration.

Secondly, BCDMH treatment had to be implemented in a way that can release specific amounts of BCDMH very quickly. The treatment had to be controllable to increments of 0.1 ppm BCDMH. The treatment also had to be safe, reliable, and repeatable. Working with a strong oxidising agent can be dangerous to the operator, and such risks must be kept low. The treatment should take effect immediately in order to be able to measure the contact time.

3.3.2 Choice of treatment

BCDMH is an organic compound that slowly releases bromine and chlorine. Hypobromous acid (HOBr) and hypochlorous acid (HOCl) becomes the active disinfecting agents, these are both well-known disinfectants used in a variety of applications. BCDMH has been on the market for a few decades, but has not been implemented widely. In South Africa, BCDMH is currently being implemented in the Aquaking water treatment system in combination with ionisation which makes the research more relevant and market related (Aquaking SA 2016). Further advantages of BCDMH is that it has been implemented as disinfectant for decades, is chemically understood, is safe and easy to work with in its tablet form, and is available on the South African market.

There were two main options to apply BCDMH in a way that met most of the requirements for the treatment. Firstly, the BCDMH tablets could be crushed into powder, or powdered BCDMH could be procured, but the rate of reaction of the powder with water and the effect of different size particles will limit the repeatability. The measuring-off of such small amounts of BCDMH, <3 mg, is impossible with the equipment available. The second option was to make a liquid BCDMH stock solution that would have constant characteristics and which would then be used as a source of BCDMH.

It was decided to make a BCDMH stock solution. From a stock solution, extremely small liquid volumes could be removed and added to the treatment process. The treatment process would then not be such a representation of the industrial system, but would comply with all the other requirements of the BCDMH treatment. The stability of a BCDMH stock solution had to be investigated as nothing could be found on literature about it.

3.3.3 Challenges with treatment

The creation of a BCDMH stock solution had two main challenges. The first challenge was to determine how strong the BCDMH stock solution could be. The second challenge was to determine how long it took for all the BCDMH to react with the water. And the third challenge was how long the stock solution remained stable in terms of its physical and chemical properties. On other words, what would be the life-span of the stock solution? The challenges were approached by first investigating all the available information on BCDMH dissociation in water and to then investigate and compare it to experimental stock solutions. For the experimental stock solution, it was decided to monitor bromine and free chlorine concentrations, ORP, pH, and EC. From the gained knowledge decisions were made regarding the use of a BCDMH stock solution for treatment.

BCDMH has a very low solubility of between 0.15 and 0.2 g/100 mL at temperatures between 20°C and 25°C (Walker, Rogers et al. 1994). A BCDMH stock solution therefore had to have a concentration below 1500 ppm, otherwise all the BCDMH will not dissolve. BCDMH has a low solubility, dissolves slowly, and has a variable rate of dissociation into HOCl and HOBr. These factors complicated the making of a BCDMH stock solution and the understanding thereof. Yeoman, Grunewald et al. (2001) developed a model for the dissolution rate of BCDMH, given in Figure 20, which estimates that about 90% of BCDMH dissolves after an hour (Yeoman, Grunewald et al. 2001). On the other hand, bromine and chlorine can be smelled with BCDMH treatment, which indicates the evaporation of chlorine and bromine species. The evaporation would indicate a limited lifespan as the bromine and chlorine available would decrease.

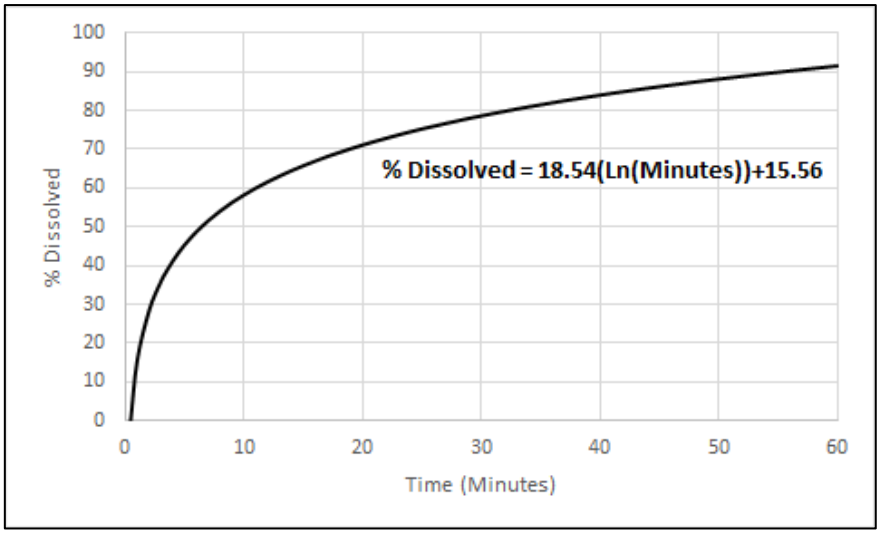

Figure 20: Dissolution of BCDMH redrawn from Yeoman, Grunewald et al. (2001)

The first few investigations were done to identify the appropriate concentration to use for a stock solution before the characteristics of the stock solution were investigated. A high concentrated stock solution is easier to make and more exact when considering the range of the scale that is used to measure-off BCDMH powder. Other advantages include that a small amount of stock solution is needed and that the feed characteristics is not changed by the addition of a large volume of water. The solubility of BCDMH supported a stock solution with a concentration of 1500 ppm BCDMH, but visual observations showed that there was still undissolved powder when it was mixed. ORP, pH and EC monitoring did not show a significant difference in measurements when comparing BCDMH solutions of 500 ppm, 1000 ppm or 1500 ppm. A stock solution of BCDMH dissolved in RO water with a concentration of 1000 ppm was chosen.

BCDMH should release HOCl and HOBr as it dissolves and dissociates in the water. A constant bromine and free chlorine concentration should therefore be an indication of complete dissolving of BCDMH. Figure 21 shows the measured bromine and free chlorine over 22 hours.

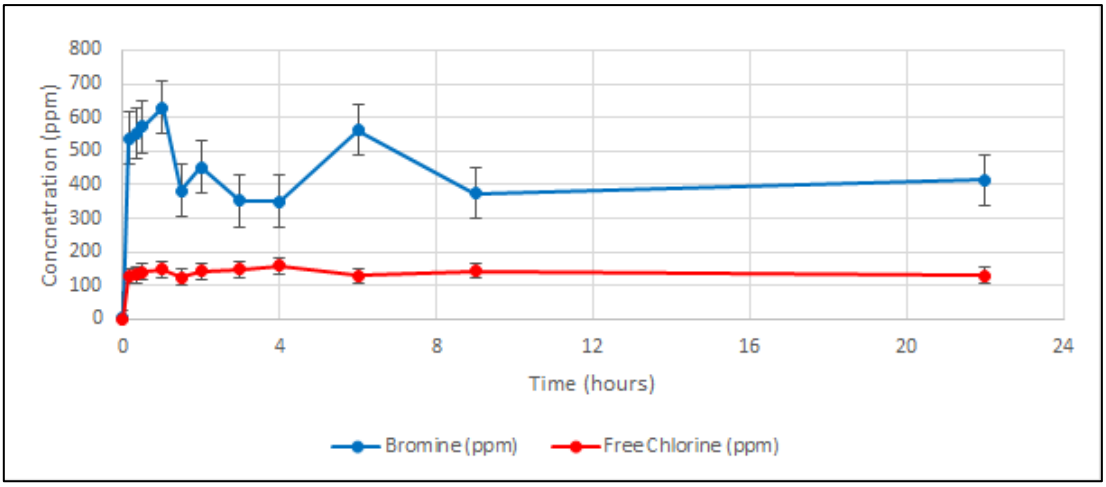

Figure 21: Free chlorine and bromine of BCDMH solution vs time

The free chlorine concentrations jumped to 140 ppm within 30 minutes and remained relatively constant for the 22 hours. The bromine measurements are displayed even though the measurements had too much of a variance. The bromine concentrations increased drastically initially, and then slowly, until it reached 630 ppm after 60 minutes. The measured bromine concentrations were unstable after the first hour. The bromine checker, used for the bromine measurements, was not very consistent when compared to the free chlorine checker. A constant bromine and free chlorine concentration was to be expected when the dissociation of BCDMH was taken into account.

Oxidation reduction potential is sometimes used to give an indication of the presence of oxidising agents at low concentrations. The ORP monitoring was used to get an indication of oxidising agent and change in reactivity of the water as the BCDMH dissociates. Figure 22 compares three different stock solutions monitored of which two were sealed and one was unsealed. Two of the stock solutions were sealed and gave similar results, which supported the ability to repeatedly make stock solutions with similar concentrations. The unsealed stock solution increased in ORP slower and levelled off at a slightly lower ORP, although the lower ORP is not necessarily significant. Both sealed solutions reached a relatively constant ORP after 600 minutes, i.e. 10 hours. An ORP of above 1000 mV was reached within about 60 minutes, but there was a continuation in reactions as the ORP continued to climb steadily. The ORP does not correlate with the measured bromine or free chlorine.

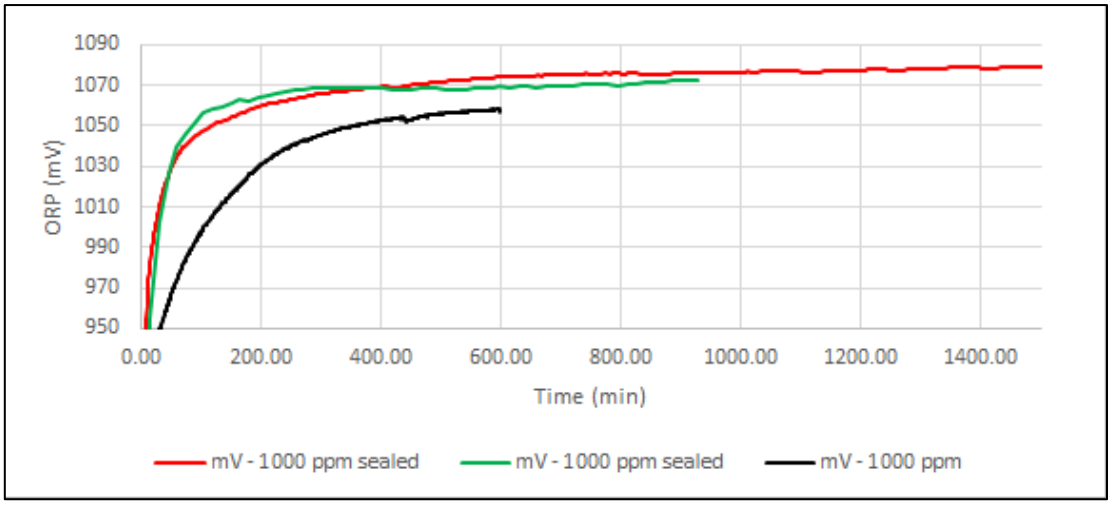

Figure 22: ORP monitoring of BCDMH stock solution

The electric conductivity (EC) of a solution is an indication of all the dissolved solids in it. When monitoring the EC of the BCDMH solutions, it was observed that the conductivity continued to increase continuously. Figure 23 shows that the gradient of the EC line is relatively constant after about 300 minutes, which means a constant increase in EC.

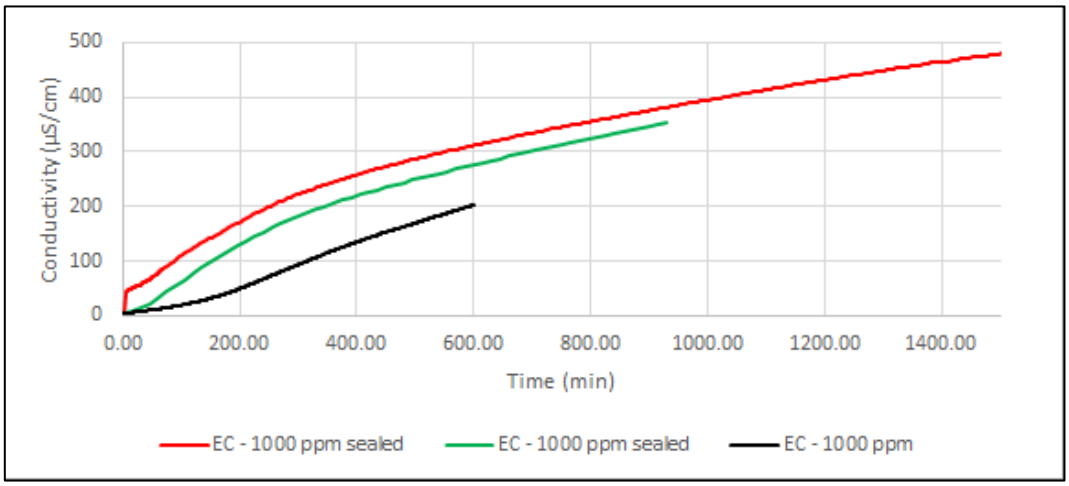

Figure 23: Conductivity monitor of BCDMH stock solution

The cause of the constant increase in EC was further investigated by looking at RO water. An investigation of RO water also showed a constant increase in EC when sealed and when not sealed. A further investigation on a BCDMH solution with periodic EC measurements showed similar results to Figure 23. The EC of the sealed BCDMH solution is higher than the EC of unsealed BCDMH solution, but the rate of increase is the same after 300 minutes and the EC continues to increase. The reason for the continuous increase in EC could not be determined.

BCDMH reacts with water to release HOBr and HOCl which are both weak acids which would decrease the pH of the solution. Figure 24 shows how the decrease in pH for the dissociation of BCDMH.

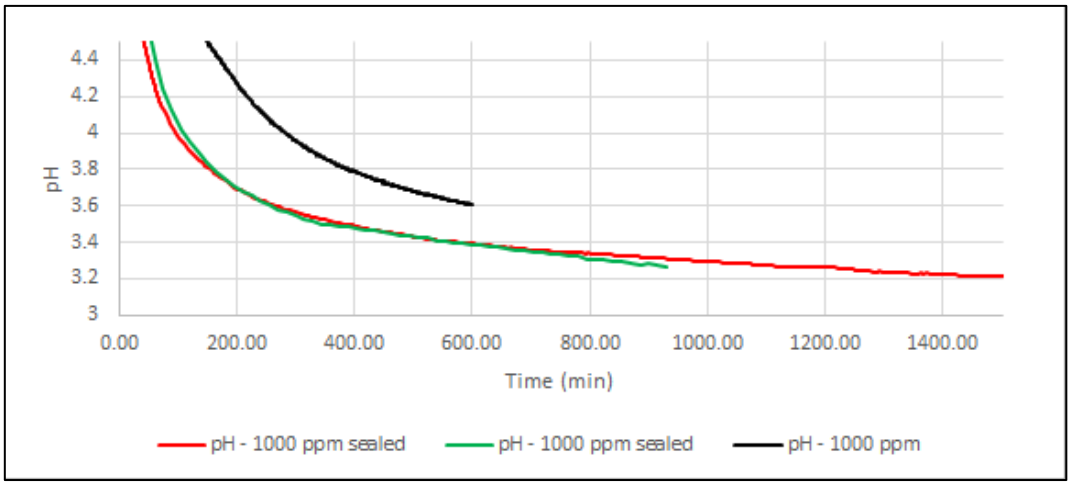

Figure 24: pH monitor of BCDMH stock solution

The decrease in pH was nearly the same for the sealed solutions, but once again the unsealed solution showed a lag in the pH decrease that is about 0.2 less than the sealed solution. The measured pH of the BCDMH solution also continued to decrease, even after 20 hours. When RO water was monitored, the pH became constant and remained relatively constant. The continuous decrease in pH is not understood, but could explain the continuous increase in conductivity. The decrease in pH requires an increase in H^+ ions which could respond to the increase in conductivity.

Although the exact reactions of the BCDMH stock solution are not understood, a few assumptions were made to get to a practical solution. It was assumed that BCDMH stock solution will have a constant oxidising ability for a duration of 12 hours from an age of 10 hours to 22 hours. This time was chosen to ensure complete dissolving of the BCDMH powder from the theoretical dissolving model, visual inspections, as well as the monitored ORP and pH. It was assumed that the increase in EC and decrease in pH is insignificant while the solution's age is kept below 22 hours. BCDMH treatment was therefore be implemented from a stock solution with a concentration of 1000 ppm that is between 10 hours and 22 hours old.

3.3.4 Protocol for BCDMH treatment

For general experimentation 50 mL 1000 ppm BCMDH stock solutions were mixed. BCDMH tablets were crushed to powder and then 50 mg BCDMH was measured-of on the scale and added to 50 mL RO water in a 100-mL glass bottle with a magnet inside and a lid. The solution was then placed on a magnetic stirrer that continuously stirred the solution. The bottle then contained 1000 ppm BCDMH solution that was deemed usable from an age of 10 hours to 22 hours.

The BCDMH treatment involved using a pipette to remove the exact amount of BCDMH solution and adding it to the contaminated water. The amount of BCDMH solution was calculated beforehand according to the required concentration of BCDMH treatment.

3.4 Metal ion treatment

3.4.1 Requirements

Part of the treatment process is the addition of metal ions, which should function as a disinfectant component into the contaminated water. The 1st objective of the research was to determine the contribution, if any, of metal ions to BCDMH disinfection. The 2nd objective was to investigate the feasibility of the combined technology and its implementation. The metal ion treatment, therefore, had to be done in a way that was comparable to industrial applications and it had to be implementable in combination with the oxidising agent, BCDMH. Factors that had to be considered were how to introduce metal ions into the contaminated water, how to control the metal ion concentrations, how to quantify the metal concentrations, and how an industrial ionisation setup would look like.

From a practical perspective, the metal ion treatment process and apparatus had to be able to be implemented on a small scale in a laboratory environment. For experimental reasons, the treatment procedure had to be repeatable and precise. To be able to make any deductions about different metal concentrations, the metals released had to be repeatedly the same and measurable. Ideally, a quick and cheap method was needed that could be used to determine the metal concentrations after treatment to analyse the effect of metal concentrations on disinfection.

3.4.2 Choice of treatment

The release of metals can be achieved through two different methods. Either stoichiometric amounts of metallic salts can be added that will release the desired amount of metal ions, or an electrolytic cell can be used to release the metal ions from the metal through the process of ionisation. The addition of metallic salts can be very precise as stoichiometric amounts of salt can be measured-off and added to the contaminated water. Metallic salts can also be used to make a stock solution, which can be used to treat water with smaller amounts of metal ions. The addition of metal salts does not, however, necessarily represent ionisation. Considering the broader aim of investigating ionisation-oxidation disinfection, this did not seem the better alternative. Although research in the past has made use of metal salts to investigate metal ion disinfection, actual ionisation was preferred for this research (Huang, Shih et al. 2008).

There are different methods of implementing, controlling, and measuring ionisation. For the research, there were three methods that were applicable/appropriate. The metal concentrations could be measured, the current applied could be measured, or the change in mass of the electrodes could be measured. The first approach, referred to as approach A, was to measure the concentration of metals in the solution after an experiment. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the metal concentrations. The second approach, referred to as approach B, was to use a basic ionisation model to calculate the theoretical number of electrons ionised from the applied current and time of ionisation. The third approach, referred to as approach C, was to measure the change in mass of the sacrificial anode.

Small scale ionisation was chosen as the method to release metal ions into the contaminated water. The ionisation was designed to represent the Aquaking ionisation treatment, as the Aquaking technology makes use of an ionisation-oxidation process with BCDMH as oxidising agent (Aquaking SA 2016). The ionisation treatment, therefore, is comparable to an industrial process currently being implemented and an improved understanding of the ionisation-oxidation should be valuable to improving the technology. A variety of different metal concentration combinations can be investigated to determine the effect every metal has on the ionisation-oxidation treatment, but this was not part of the scope of the research.

The ionisation was done by making use of copper-silver-zinc alloy electrodes that were connected to a variable direct current (DC) power supply. The copper-silver-zinc electrodes were cast according to the Aquaking specifications in the ratios of their technology (Aquaking SA 2016). The variable power supply was used to supply a fixed current to control the amount of metal ions released. Ionisation was controlled and measured by the theoretical number of electrons, in coulombs, ionised by the applied current per litre volume. Measuring the applied current and time of ionisation proved to be simpler and more repeatable than measuring the actual metal concentrations or measuring the change in electrode mass. The choice is explained in detail in *3.4.3.5 Measuring ionisation*.

3.4.3 Challenges with treatment

3.4.3.1 Theoretical ion release

The release of metal ions through ionisation is a simple chemical process, which can become complex depending on the water content. An electric current is a flow of electrons through a conductor. There are different forms of electrochemical cells, some require an applied voltage, while others cause a flow of electrons. An electric source can be applied to electrodes in a solution that forms an electrochemical cell as in Figure 25. An electrochemical cell cause oxidation and reduction half reactions to take place because of the applied potential. For ionisation, electrons are released at the

cathode where reduction take place, and electrons are removed at the anode where oxidation takes place. The metal anode becomes a sacrificial anode that releases metal ions into the water as the applied power source oxidises the metal to positive metal ions.

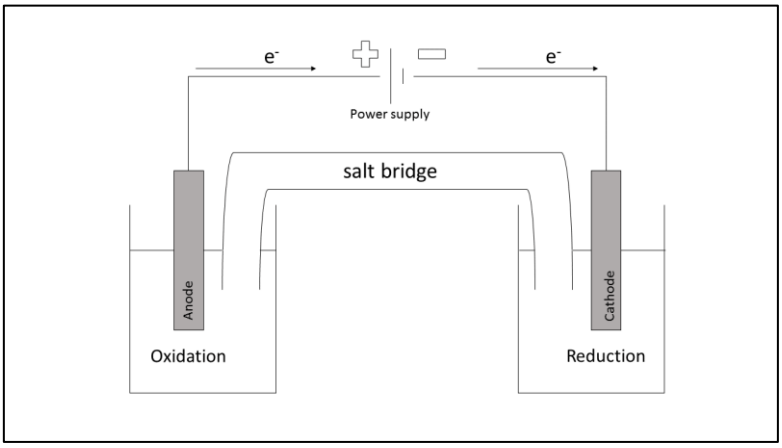

Figure 25: Basic electrochemical setup

To understand the influence of metal ion concentration on bacterial disinfection, metal ion release needs to be monitored and controlled. It is not always practical and financially viable to measure the metal concentrations for every experiment and therefore alternative approaches are required to measure ionisation. A basic ionisation model was developed to be able to compare the measured metal concentrations with the applied current, and with the changes in anode mass. Although such models already exist, it was decided to develop a model from basic principles of physics and chemistry. The model was built on the idea that an applied electric current will release a certain mass of metal ions which will result in a specific metal concentration. The model was simplified to assume all the electrons removed from the anode released metal ions and that metal ions were released in the same ratio that they were present in the anode.

The model was built from a few basic principles. At the cathode electrons were released, which were irrelevant for the ionisation, but at the anode electrons were removed which caused metal ions to be released. Current is measured in ampere (A) and 1 ampere is defined as 1 Coulomb of electrons that pass a point in a conductor per second, Equation 19. A mole of electrons is defined as a Faraday and 1 Faraday is equivalent to 96 485 Coulomb electrons, Equation 20. Therefore, a current that runs through an electrochemical cell for a time (Δt) will cause the release of several coulomb electrons at the cathode and the removal of several coulomb electrons at the anode. The number of electrons ionised ($C_{electrons}$), measured in coulomb, can be calculated using Equation 21. The electrons ionised can also be calculated in mole, Equation 22, which can then be used to calculate the mole metals released.

$$1\ Amp = 1\ Coulomb/sec \quad \text{(Eq. 19)}$$

$$96485\ Coulomb = 1\ Faraday = 1\ mole\ of\ electrons \quad \text{(Eq. 20)}$$

$$C_{elctrons} = I\Delta t \quad \text{(Eq. 21)}$$

$$n_{elctrons} = \frac{I\Delta t}{96485} \quad \text{(Eq. 22)}$$

Where:

- $C_{electrons}$ = electrons ionised in coulomb;
- $n_{electrons}$ = mole electrons ionised in mole;
- I = current in ampere;
- Δt = time of ionisation in seconds.

The model assumes that the mole electrons ionised will release an amount of metal ions according to the make-up of the anode and the number of electrons a metal releases when oxidised, i.e. oxidation number. The mole electrons ionised are, therefore, equivalent to the sum of the mole of metals released multiplied by their oxidation numbers, Equation 23.

$$n_{elctrons} = \sum_{j=1}^{k} O_j n_j \quad \text{(Eq. 23)}$$

Where:

- $n_{electrons}$ = mole electrons ionised in mole;
- n_i = mole of metal i released;
- O_i = oxidation number of metal i;
- k = number of different metals.

By making use of the fact that the mole of a metal is equal to its mass divided by its molar mass, Equation 24, and the assumption that the mass of a specific metal oxidised is equal to its percentage of the alloy multiplied by the all the mass multiplied, Equation 25, a relationship can be derived between the electrons ionised and the mass of the anode lost, Equation 26.

$$n_i = \frac{m_i}{M_i} \quad \text{(Eq. 24)}$$

$$m_i = \%_i m \quad \text{(Eq. 25)}$$

$$n_{elctrons} = m \sum_{j=1}^{k} \frac{O_j \%_j}{M_j} \quad \text{(Eq. 26)}$$

Where:

- $n_{electrons}$ = mole electrons ionised in mole;
- n_i = mole of metal i released;
- m_i = mass of metal i released in gram;
- M_i = molar mass of metal i in gram/mole;
- $\%_i$ = percentage of electrode that consists of metal i;
- m = total mass of anode lost due to oxidation;
- O_i = oxidation number of metal i;
- k = number of different metals.

Equation 4 and Equation 8 can be combined to create a relationship between the applied current and the mass of metal lost from the anode due to ionisation, Equation 27.

$$m = \frac{I \Delta t}{96485 \times \sum_{j=1}^{k} \frac{O_j \%_j}{M_j}} \quad \text{(Eq. 27)}$$

Where:

- m = total mass of anode lost due to oxidation;
- I = current in ampere;
- Δt = time of ionisation in seconds;
- M_i = molar mass of metal i in gram/mole;
- $\%_i$ = percentage of electrode that consists of metal i;
- O_i = oxidation number of metal i;
- k = number of different metals.

To determine the concentration of a metal from ionisation, the mass of the metal can be divided by the volume of the liquid, Equation 28.

$$c_i = \frac{1000 \times m_i}{V} \quad \text{(Eq. 28)}$$

Equation 25, Equation 27, and Equation 28 can be combined to create a relationship between the applied current and the concentration of the different metals due to ionisation (Equation 29).

$$c_i = \frac{I\Delta t\%_i}{96.485 \times V \sum_{j=1}^{k} \frac{O_j \%_j}{M_j}} \quad \text{(Eq. 29)}$$

Where:

- c_i = concentration of metal i in ppm;
- m_i = mass of metal i in gram;
- V = volume of solution in litres;
- I = current in ampere;
- Δt = time of ionisation in seconds;
- M_i = molar mass of metal i in gram/mole;
- $\%_i$ = percentage of electrode that consists of metal i;
- O_i = oxidation number of metal i;
- k = number of different metals.

The models and Equations that were derived are limited, and simplistic, due to the following assumptions made in the development thereof:

- The applied current gains all the electrons from the metals becoming ions.
- Only metals are reduced to metal ions and no other reactions take place at the anode.
- The oxidising potential of the different metals don't influence the ionisation.
- The metals are released in the same ratio as the anode is made up of.
- The metals will remain in solution and not precipitate immediately.
- No metal ions are removed at the cathode.

Water content was not considered, but would complicate ionisation because depending on the different salts the anode will not be oxidised, but other reactions will take place. It was decided to make the model acceptable for EC of below 150 µS/cm. The effect of the different oxidation potentials of the different metals in the alloy on metal release were also not considered. Zinc has the highest oxidising potential and should therefore oxidise first, followed by copper, and then silver (Vanýsek 2012). This model assumed that the metals will be released in the same ratio as the make-up of the anode. Other factors not included in the model were movement of water, surface area of anode available for ionisation, and current pathways in the solution.

It was earlier mentioned that the metal concentrations can be measured, or the applied current and duration of ionisation (Δt) can be measured, or the change in mass of the anode can be measured. By using Equation 27 and Equation 28, or inverses of them, the different measurements of ionisation could be compared to each other to determine the final controls employed to measure ionisation.

3.4.3.2 *Initial ionisation experiments and problems*

The first problem was to investigate whether metal ions could be released by such a simplistic electrochemical cell and whether the metal concentrations could be measured. An electrochemical setup was designed for ionisation with alloy electrodes. The electrodes composition were manufactured according to the Aquaking patent 2009/4991 with permission from Aquaking (Aquaking SA 2016). The schematic design can be seen in Figure 26 and the picture thereof in Figure 27. The average current was measured using a Fluke 179 multi-meter, the electrodes' weight was measured using a scale, and the metallic concentrations were measured using the ICP-MS analysis. Equation 27 and Equation 29, and derivatives thereof, were used to investigate the different approaches to measuring ionisation.

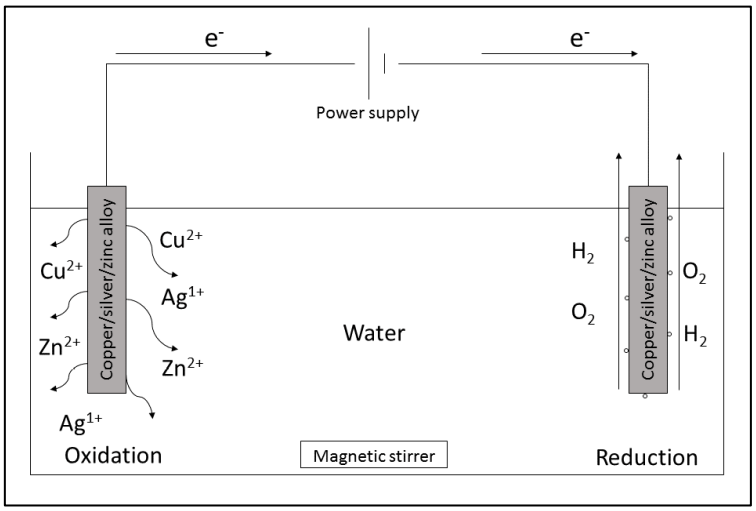

Figure 26: Ionisation setup for copper, silver and zinc ionisation

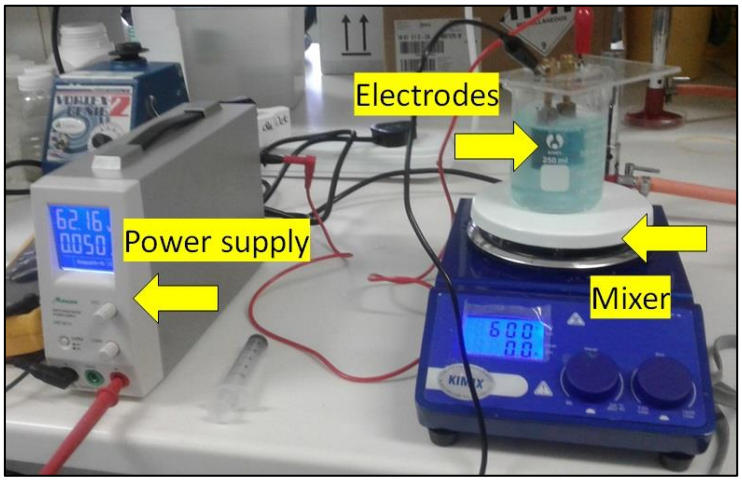

Figure 27: Picture of ionisation setup in laboratory

The first ionisation experiments revealed some of the challenges that entailed measuring ionisation. The measured concentrations is displayed on figure 28.

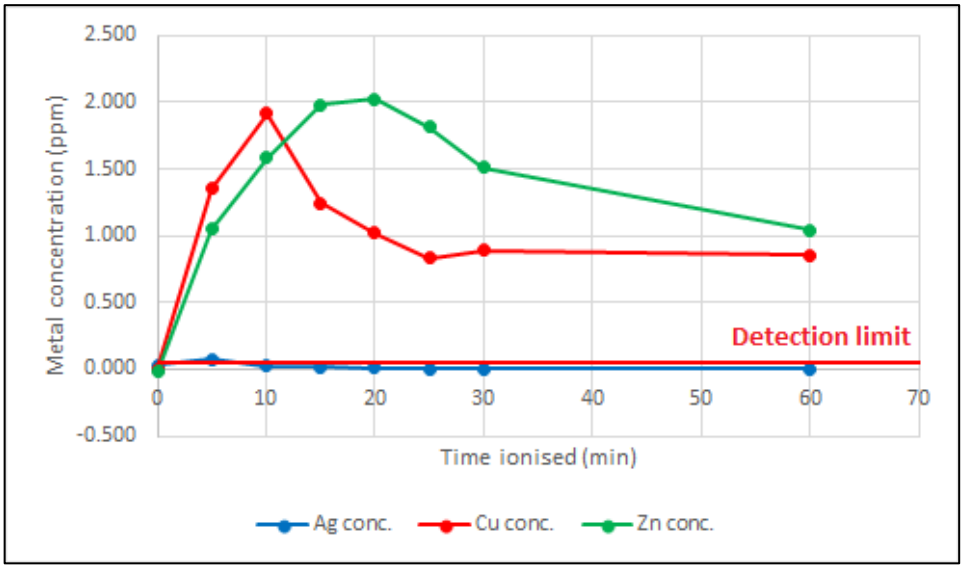

Figure 28: Initial experiments of measured metal concentrations vs time ionising

Copper and zinc concentrations were detectable, and increased initially to approximately 2 ppm, before suddenly decreasing to concentrations of approximately 1 ppm and remaining constant. Silver concentrations, on the other hand, were predominantly below the detection limit of 0.001 ppm. The measurements of the electrodes' mass showed that the anode was being oxidised, as it decreased in mass, whilst the cathode mass remained the same. The relationship between the change in anode mass and current applied were similar to the projected model. Visual observations of reactions showed filament structures around the anode, a change in colour of the solution and gas bubbles around the cathode. These observations supported the idea of metal ions being released at the anode and other gases, probably O_2 and H_2, forming at the cathode.

3.4.3.3 Metal precipitation

The decrease in metal concentrations were attributed to the precipitation of metal salts. The 0.22 µm filters used to remove particles out of samples before the ICP-MS analysis quickly became blue and clogged. Nitric acid was used to investigate the possibility to dissolve the metal salts before ICP-MS analysis. The addition of nitric acid caused copper and zinc to dissolve, while silver was still hardly detectable. Figure 29 shows how the metal complexes dissolve as nitric acid is added. The measured copper and zinc concentrations after the addition of nitric acid became comparable to the theoretical expected concentrations calculated from the current applied and change in mass of the anode. The measured zinc concentrations were generally more than expected from the ionisation model and the copper concentrations were slightly less than expected concentrations from the model.

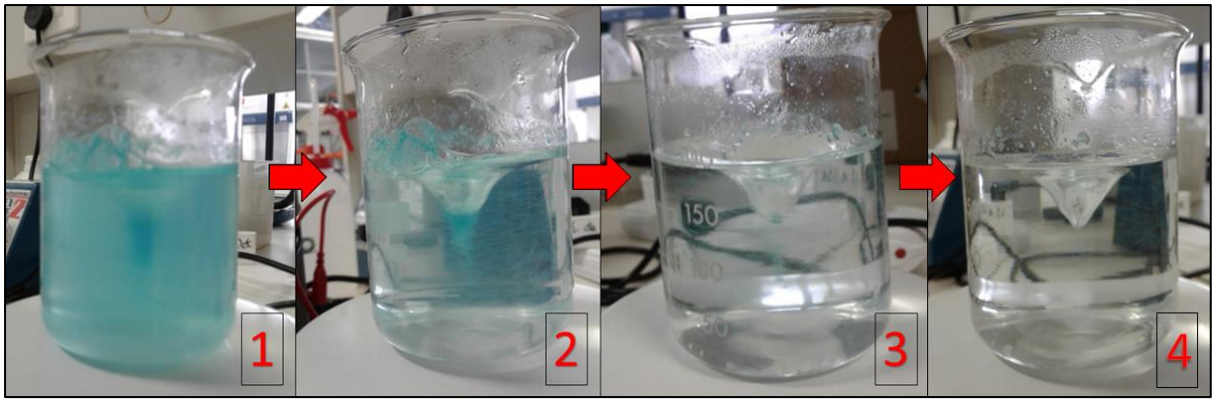

Figure 29: Metal complexes dissolving with the addition of nitric acid

Literature was investigated to gain a better understanding of the precipitation of metals. Previous ionisation-oxidation research also mentioned the formation of insoluble copper complexes (Lin, Vidic et al. 2002). Each metal has a concentration limit after above it will precipitate, depending on the pH, temperature, and other constituents in the water. The complexity of the reactions can be understood by looking at copper ions. Figure 30 illustrates the copper speciation at different pH and Figure 31 illustrates the precipitation of copper hydroxide. When developing a water disinfection process, it must be functional on any water source that can be used as drinking water and therefore the exact precipitation kinetics are not essential and beyond the scope of this research. The metal ion speciation could influence disinfection, but a more precise approach would be needed to investigate it. The repeatability of metal release, and which metals are released are of more value.

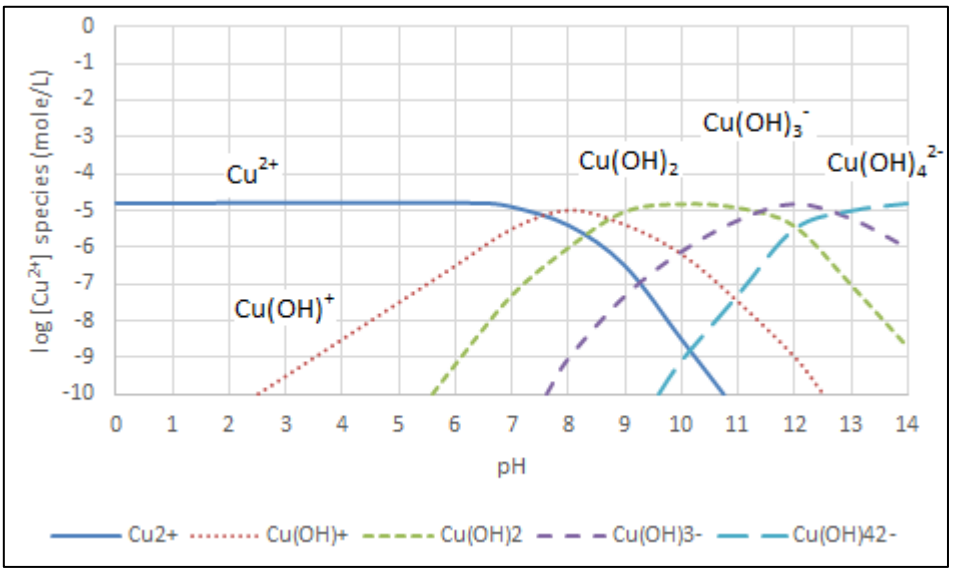

Figure 30: Copper speciation vs pH redrawn from Cuppett, Duncan et al. (2006)

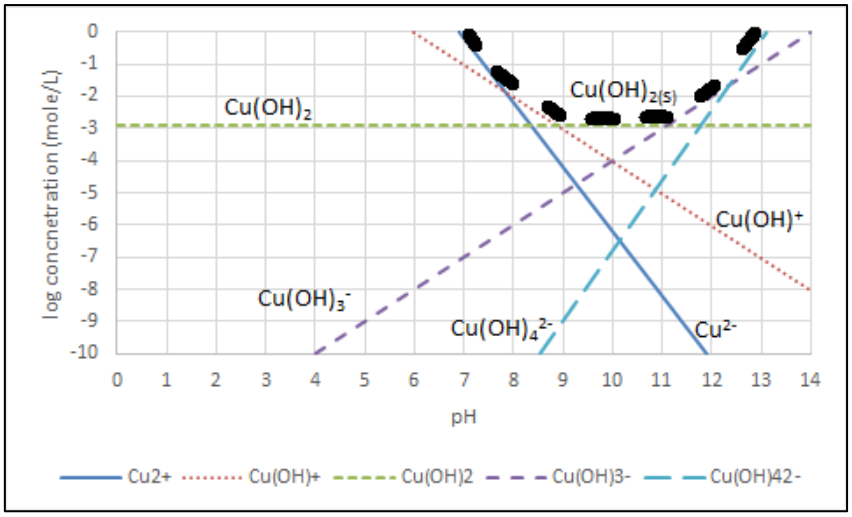

Figure 31: Copper hydroxide precipitation redrawn from Cuppett, Duncan et al. (2006)

3.4.3.4 Determining silver ionisation

The absence of silver in the ICP-MS analysis was problematic, since silver is the strongest disinfectant of the three metals. There were different possible reasons for this dilemma. Firstly, there was the possibility that the electrodes did not contain silver. Secondly, the silver was perhaps not oxidised because zinc and copper are oxidised more readily than silver. The silver would then only be oxidised at a later stage, or could be released as silver particles as the copper and zinc are ionised around it. The third possibility was that the silver precipitated very quickly and that nitric acid could not dissolve it, and therefore it was removed by the 0.22 µm filtration. Two different experiments were done to investigate silver release. First ionisation was done with a pure silver anode and then a second experiment was done with a combination of three separate anodes, one silver, one copper and one zinc.

Two experiments were done to determine silver release when a pure silver anode is used for ionisation. The first experiment revealed that the detectable silver concentrations remained extremely low and then suddenly started to increase after 15 minutes of ionisation. The solution became a murky white colour and the addition of nitric acid did not cause the solution to clear up. The change in mass of the anode revealed that the silver concentrations had to be much higher than what was measured through ICP-MS analysis. A second ionisation experiment with a pure silver anode was done and the anode was dried and weighed between every sampling. Figure 32 compares the measured silver concentrations with the change in mass of the silver anode. The silver, once again, remained nearly undetectable until 20 minutes of ionisation, after which its measured concentration increased quickly. The change in anode mass was comparable to the theoretical change in mass calculated from the applied current and shows that silver was released continuously. The increase in

silver concentration was, however, not detectable by the ICP-MS analysis. From these experiments it was clear that silver concentrations were not quantifiable by the available technology.

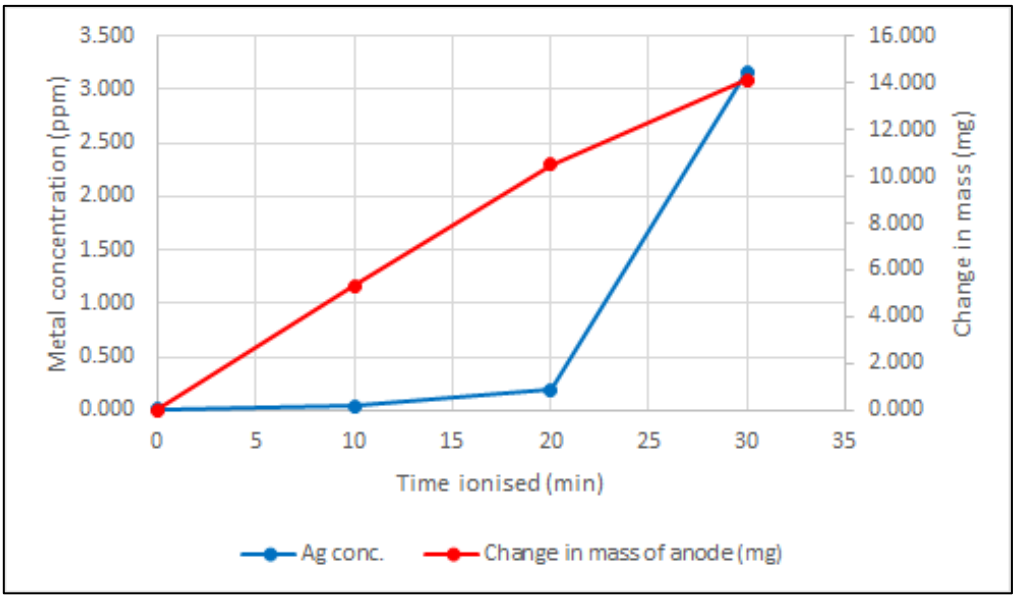

Figure 32: Measured silver concentration vs change in silver anode mass

To have a reference to compare the silver anode ionisation with, pure copper and zinc anodes were also investigated. The copper concentrations measured were about 86% of the theoretical copper concentrations expected due to the applied current. The measured concentrations were 1.5 times more than what was expected from the measured change in mass of the copper anode. The zinc ionisation was the closest to ideally represent the equations developed earlier. Figure 33 shows the different approaches to determine the change in mass for ionisation.

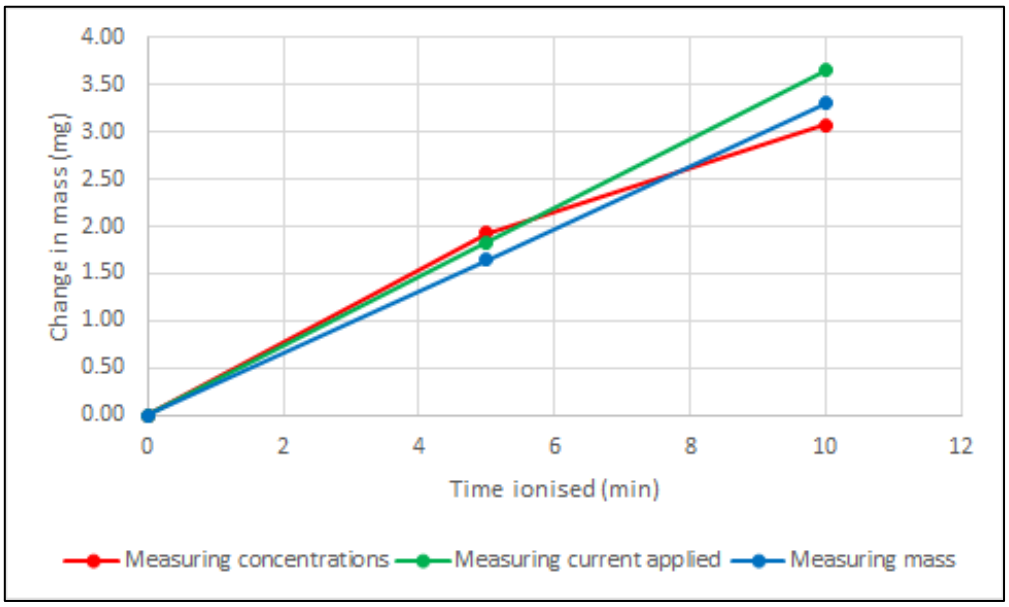

Figure 33: Calculated change in mass from different measurements for ionisation with a zinc anode

The measured change in mass (blue line), was similar to the change in mass calculated from the measured concentrations (red line), and similar to the change in mass calculated from the applied current. These two experiments showed that the ionisation equations developed earlier can be valuable and that although silver was not detected in the earlier experiments, it was ionised.

It was clear that silver concentrations could not be determined by the ICP-MS analysis, but it was still unclear whether silver was being ionised with the alloy anode. A special anode combination was then investigated with the three metal anodes connected in parallel. The three electrodes in parallel represented an alloy, but the individual metal electrodes could be weighed to be sure each metal was being ionised. The results showed that the silver, copper, and zinc anodes decreased in mass, therefore representing metal release. In Figure 34 the measured metal concentrations are compared to the calculated concentrations from the change in anode mass. Zinc concentrations were about 90% of the expected concentrations, copper concentrations were about 70% of the expected concentrations, and silver concentrations were again not detected. From the ionisation experiments that showed the decrease in silver anode mass, it was concluded that silver was ionised from the alloy electrodes although the silver was not detected by ICP-MC analysis.

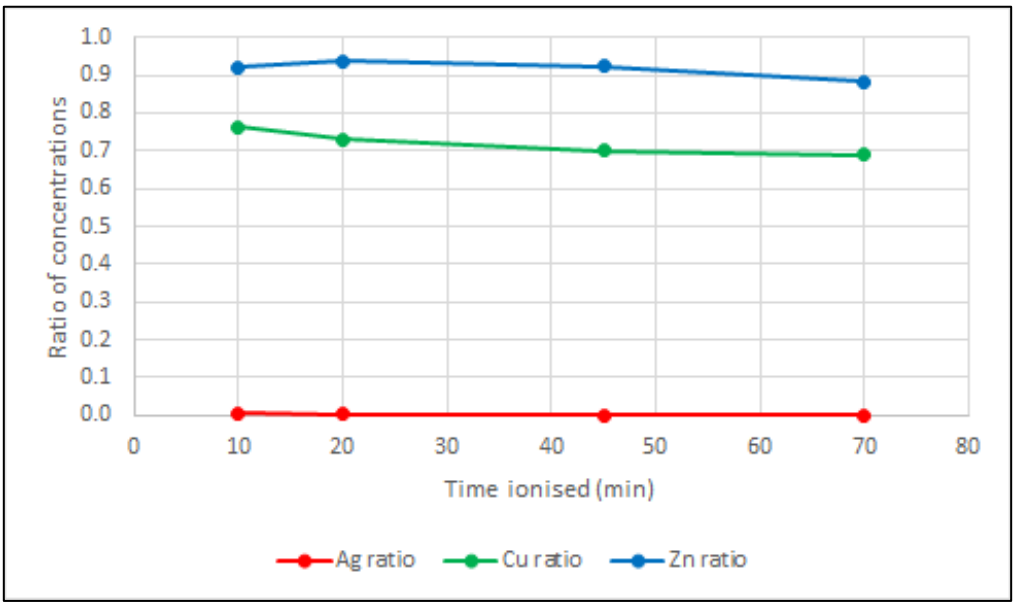

Figure 34: Ratio of measured metal concentration compared to the calculated concentrations from the change in mass

3.4.3.5 Measuring ionisation

From the investigation into silver ionisation, it was clear that exact metal concentrations could not really be determined. Therefore, final disinfection would also not be comparable to actual metal concentrations, but one of the other measurements for ionisation had to be used. From experiments the measured change in anode mass and the measured applied currents were often comparable.

Measuring the anode mass, however, had some discrepancies. To weigh the anode, the anode had to be dried and this caused a thin layer of suspended metal particles to be removed out of the solution. The ionisation system also had to be disassembled to be able to weigh the electrodes, and it took extra time to do this. The measuring of the applied current, on the other hand, was quick and easy as the Fluke 179 multi-meter calculated an average current. It was therefore decided to measure ionisation in terms of the coulomb electrons released per litre volume calculated by multiplying the applied current with the time of ionisation (Δt) and dividing it by the volume, Equation 30.

$$C/L_{elctrons} = \frac{I \times \Delta t}{V} \quad \text{(Eq. 30)}$$

Where:

- $C/L_{electrons}$ = electrons ionised in coulomb per litre volume;
- I = current in ampere (A);
- Δt = time of ionisation in seconds;
- V = volume of solution in litre (L).

3.4.3.6 Repeatability

Part of the challenge with the ionisation experiments were repeatability. Although it was decided to measure ionisation in the coulomb electrons released, ideally the amount of metal concentrations released should be similar for similar experiments. Three experiments were done with tap water investigating an applied current of ± 18 mA with alloy electrodes in tap water. Samples were taken after 5 minutes and after 10 minutes ionisation. As seen in Figure 35, the metal concentrations varied little, while silver was not detectable. The measured metal concentrations were, on average, 86% of the theoretical metal concentrations calculated from the applied current after 5 min and 79% after 10 min ionisation. 5-minute experiments showed repeatable metal release.

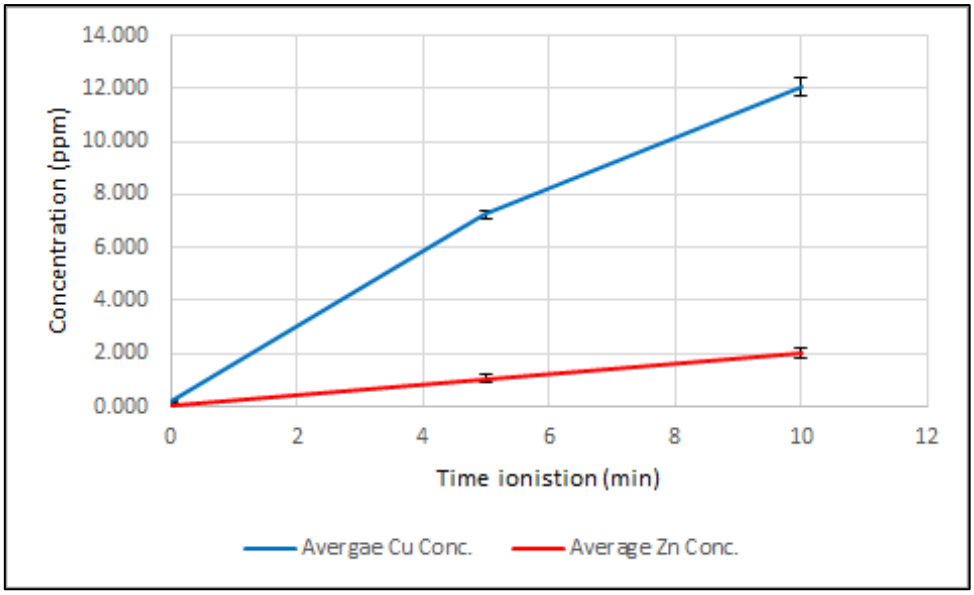

Figure 35: Repeatability of copper and zinc concentrations measured for time ionised

3.4.3.7 Other ionisation factors

Other water factors were investigated to see the effect on ionisation. Ionisation of water with salt added (2g/L) did not release detectable concentrations of metal ions initially. Only after 20 minutes the metal concentrations increased rapidly. Ionisation of water with bentonite (2 g/L), representing mud particles, showed different ionisation characteristics. Metal concentrations were much lower than expected compared to the change in mass of the anode. The metal particles formed stable colloids with bentonite particles due to charge stability and low zeta potential. These colloids were then removed by the 0.22 µm filters. A carbon cathode instead of an alloy cathode yielded similar metal release from the anode. New alloy electrodes showed the closest relationship between the three methods of measuring ionisation. Ionisation could not be monitored continuously by ORP, pH or EC, due to the electric current. Generally, ionisation caused a noticeable decrease in the ORP of any solution.

3.4.3.8 Final preliminary ionisation investigation

A final, longer ionisation experiment was done in 900 mL water, which was to be comparable to ionisation for the treatment experiments. Although ionisation was to be conducted with the release of low metal concentrations, the low concentrations of below detection limit of 0.001 ppm could not be detected. The investigation, therefore, focused on a longer time period of 70 minutes to see whether the process continued the same and to compare it to industrial applications. The ionisation went similar to previous ionisation experiments. The addition of more nitric acid with the final sample did not make a significant difference in metal concentrations. The three measurements of ionisation are compared for the full ionisation time in Figure 36. The measured change in mass of the anode was

slightly higher than the calculated change in mass from the measured concentrations and the measured applied current. The trends followed by all three measurements show that the ionisation pattern did not change over a 70-minute period.

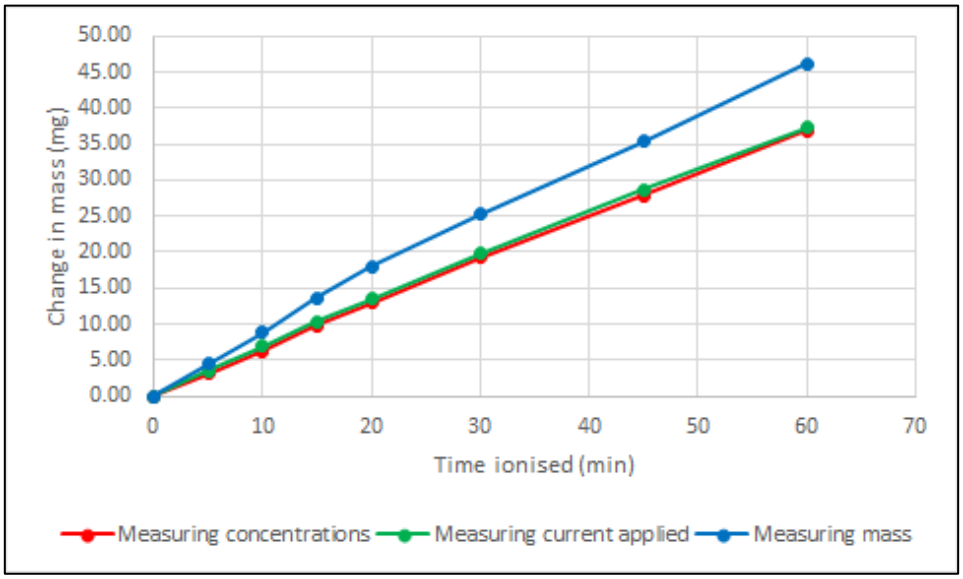

Figure 36: Measured change in mass of anode vs time ionised

The measured silver, copper and zinc concentrations were compared to the theoretical concentrations calculated by the measured current applied. Figure 37 summarised the relationships in ratios over the 60-minute ionisation time.

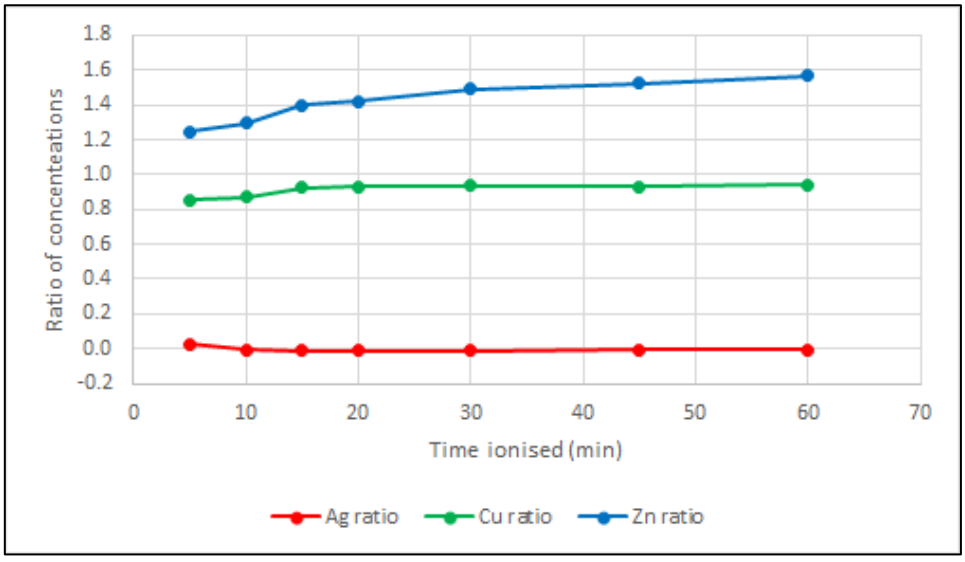

Figure 37: Ratio of measured metal concentrations vs the theoretical metal concentrations calculated from applied current for time ionised

Silver was not detected, therefore the measured percentage of what was expected is given as 0%. Copper concentrations measured were lower than the model predicted. Initially, copper

concentrations were 85% of what was expected, but increased and stabilised at about 93% of the expected concentrations. Zinc concentrations were above what was expected. The zinc concentrations were initially about 125% of what was expected and increased continuously to about 155% of the projected concentration. The different oxidising potentials of the metals are one of the explanations given to understand these observed relationships. Zinc is oxidised the easiest to zinc ions, followed by copper and then silver. The stability of metal complexes could also have influenced measured concentrations.

It was decided that coulomb electrons ionised were to be used to measure ionisation, due to reasons discussed in *3.4.3.5 Measuring ionisation*. Figure 38 compares the different methods of calculating the electrons ionised from the measured data.

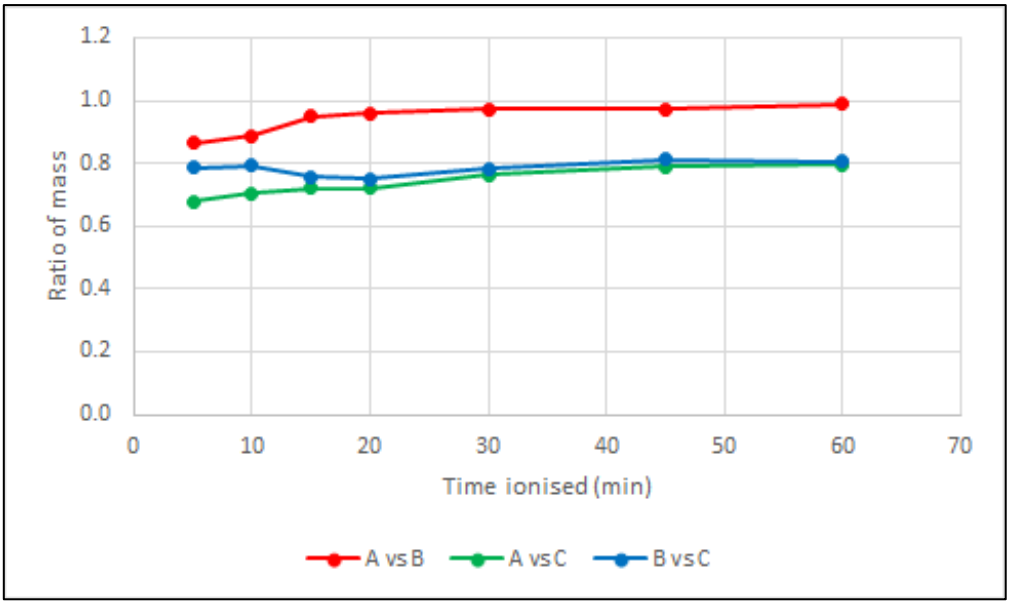

Figure 38: Ratios comparing ionisation measurements

In figure 38 A refers to calculations using the measured metal concentrations. B refers to calculations using the measured average applied current. C refers to the calculations using the measured changed in anode mass. The measured metal concentrations and applied current gave nearly the same coulomb electrons ionised. The measured change in anode mass gave some 25% more electrons ionised. It was accepted that taking all the variability of experiments and results into account, the coulomb electrons released calculated through the applied current remained the most reliable approach to measure ionisation.

3.4.4 Protocol for treatment

An electrochemical cell was used to release copper, silver, and zinc ions into the solution that was treated. A variable direct current power supply was connected to metal alloy electrodes and the

applied current then caused the release of copper, silver and zinc ions (ionisation). The ionisation was measured by the number of ions released in coulomb calculated from the applied current and time of ionisation. The ionisation regime was determined to keep treatment within a practical and safe range of treatment, i.e. the applied current was kept below 0.1 A and the voltage was kept below 60 V.

A Manson NSP-6016 variable power supply was used to supply the electrochemical cell with a fixed current. A Fluke 179 True RMS Multi-meter was used to measure the average current applied for time of ionisation. 60-gram (±5 gram) alloy electrodes were used which were manufactured by Aquaking according to the Aquaking copper-silver-zinc relationship. The power supply had three current settings at which it could operate when applied to ionisation in tap water: ±16 mA, ±27 mA and ±38 mA. The ionisation was applied for lengths between 1 and 5 minutes.

Treatment was conducted to keep metal concentrations below levels that could have negative effects on human health or the environment. Copper concentrations above 2 ppm are said to be unacceptable in potable water (SABS 2011, SABS 2015). 7 coulomb electrons ionised per litre were determined to cause a copper concentration of about 2 ppm. Average current applied and time ionised were therefore investigated in combinations that kept the number of electrons ionised below 7 coulombs per litre.

3.5 Assessment and control of disinfection

3.5.1 Requirements

Part of investigating disinfection technology, is the requirement to be able to determine disinfection. Every ionisation-oxidation treatment experiment had to be assessed to determine whether disinfection was successful. The treatment, therefore, had to decrease the bacterial loading of the feed to insignificant levels to be deemed “successful disinfection”. A control had to be in place to measure the success of the disinfection. The control had to yield repeatable results, had to be practical to measure, cheap to implement, and did not have to be too time consuming. The control had to be a good indicator of disinfection success for the treatment of *CT07*.

From literature, it was evident that there is a gap in developing hands-free continuous monitoring controls for disinfection. One of the objectives was to evaluate ORP as an indicator of disinfection efficacy. For this reason, ORP and other monitors had to form part of the assessment and control of the disinfection procedure. These results could help shed light on the applicability of alternative monitors to control and assess disinfection.

3.5.2 Choice of assessment

As discussed earlier in the literature, there are several methods of determining bacterial concentrations. Some of these methods are more expensive, and others are more time consuming. The most practical method to accurately determine treatment success was plating. Plating could be implemented in two different ways. Either bacterial log reduction or bacterial presence could be investigated. Other tests used to determine bacterial concentration or presence, such as Colilert, were deemed either too expensive or not implementable in the work space available.

It was decided to use plating to determine the presence of bacteria, i.e. the presence of *CT07*. Bacterial plating is a very quick and easy test to investigate bacterial presence. Plates are easy to prepare, relatively cheap and easy to implement in the microbiology laboratory. After experiments were conducted, samples were plated immediately without additional exposure to disinfectants and after two days the plates were investigated to determine treatment success.

Oxidation reduction potential (ORP) was the main alternative control chosen to investigate disinfection success. ORP has become a popular alternative to monitor chorine based disinfection technology. Improved understanding of ORP and its potential to assess and control disinfection will, therefore, be of value. There is no literature that describes the implementation of ORP to assess disinfection for a combined oxidation-ionisation treatment system. Other water characteristics, namely pH, electric conductivity (EC) and bromine residual were also chosen to be monitored to help to understand changes in ORP.

3.5.3 Problems with assessment

Plating has several advantages, but it also has its limitations as a tool to assess disinfection. Plating remains an estimate of the bacterial concentration and is not an exact value. For the purposes of the experimentation, the estimate was more than sufficient. Plating on TSA allows other microbe growth that can influence the growth of the *CT07* under investigation, but good aseptic techniques should prevent this. Bacteria is not necessarily spread homogenously throughout the water that is treated and therefore, samples can be a poor indication of bacterial concentrations. To ensure valid sampling, the contaminated water was stirred continuously, and all plating was done in duplicate with samples taken from different regions of the container. Plating cannot be used to determine bacterial presence when ten or less colony forming units (cfu) are identified per plate, i.e. a bacterial concentration of 100 cfu/mL or less will be undetectable.

Using plating to determine bacterial log reduction could have been a more accurate application to investigate disinfection effectiveness. Disinfection would then not have been either “successful” or

"unsuccessful", but measured to a degree of being successful. It was decided to rather use the binomial approach because of financial and time constraint, as full dilutions and plating would have been time intensive after every treatment experiment. The samples would, theoretically, still have been exposed to the biocides during dilutions and therefore the contact time would not have been controlled. Practically, water must pass a certain quality test, which then either qualifies the water as potable or non-potable. Similarly, the presence or absence of bacteria in the plating test deems treatment either as successful or unsuccessful.

Measuring ORP can be challenging. ORP probes can take several minutes to give a constant reading for a water sample. When there are still chemical reactions taking place within the sample, it is impossible to know whether the ORP readings are an indicator of the reactions taking place or whether the readings are evening out. Ideally, ORP probes should be given a few minutes to settle when placed in a new solution. When the water was treated with BCDMH, there was no time for the ORP readings to become constant after the ionisation treatment because it would influence the disinfection contact time. Therefore, the ORP monitoring was not always given enough time to even out.

3.5.4 Protocol for disinfection assessment

Tryptic Soy Agar (TSA) plates (3 g TSB/L and 15 g Agar/L) were prepared a week before experimentation. Plates are kept sealed and sterile. After treatment, 110 µL is removed from the treated solution with an automated pipette. The automated pipette is used to drop 10 droplets of 10 µL each on the petri dish under a Bunsen burner. A second sample is removed and plated. The petri dish is left to incubate at room temperature for 3 days. After 3 days the plate is scanned for any microbial growth. An average of more than 10 colony forming units (cfu) of bacteria between the two plated samples will indicate bacterial presence and unsuccessful disinfection. An average of 10 or less cfu per sample will indicate bacterial absence and successful disinfection (Huang, Shih et al. 2008).

To summarise, successful disinfection was defined as the reduction of CT07 from a concentration between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/ml, to below detection limits on a petri dish, i.e. 10 cfu/dish which is equivalent to 100 cfu/ml. Successful disinfection is therefore equivalent to about a 5-log reduction in CT07.

ORP was measured continuously with a Hanna Instruments edge® pH meter and HI36180 ORP probe. The pH was measured continuously with a Hanna Instruments edge® pH meter and HI11310 pH probe. The electrical conductivity was measured continuously with a Hanna Instruments edge® EC meter and HI763100 EC probe. The ORP, pH, and EC of the tap water was measured as the bacterial feed was

added, then stopped for the ionisation period, and then continued for the BCDMH treatment. A Hanna Instruments Bromine Clicker was used to determine bromine concentrations after treatment.

3.6 Apparatus

3.6.1 Requirements

The apparatus was required to represent an industrial oxidation-ionisation treatment process as far as possible. The apparatus had to be a small enough to be implemented in a laboratory environment without requiring unnecessary space or requiring excessive time to run experiments. The down-scale from an industrial application to a lab-scale process needed to be done in a way that would have a limited influence on disinfection.

The apparatus had to be a setup on which batch experiments could be done. These batch experiments had to have a consistent feed and consistent controls. The batch experiments had to allow for a variable amount of metal ion treatment and BCDMH treatment. Experiments had to be repeatable keeping temperature, volume and fluid dynamic conditions constant.

3.6.2 Experimental setup

The experimental setup can roughly be divided into three sections, firstly the monitoring section, secondly the treatment or process section, and thirdly the sterile microbiological section. Practically, these sections are inter-related, but can be separated because they serve different purposes. Figure 39 is a schematic diagram of the complete experimental apparatus. The Appendix A contains a section that discusses all the equipment, consumables and chemicals used and their specifications.

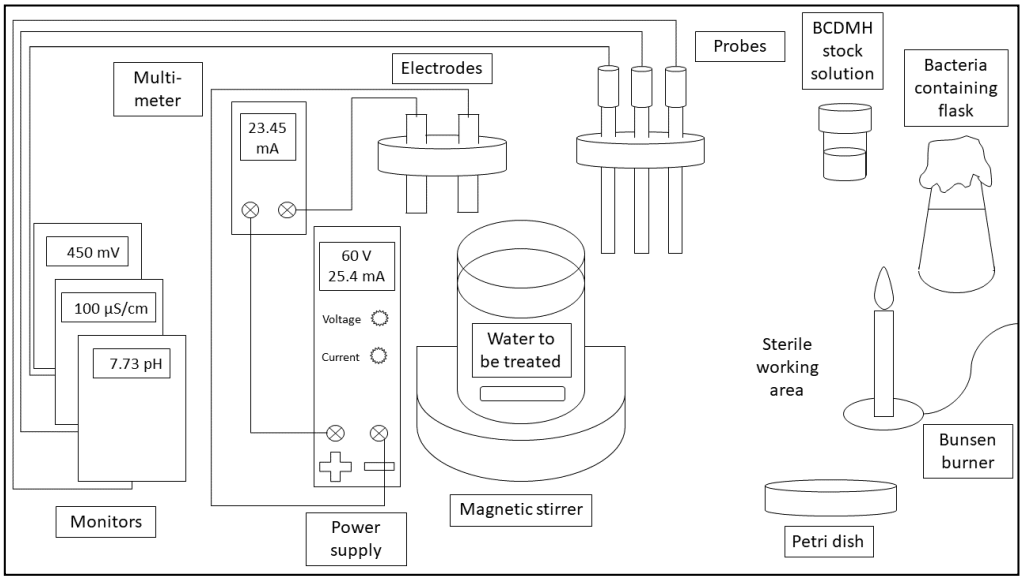

Figure 39: Schematic sketch of treatment apparatus

The monitor section consisted of the probes and meters that monitored and assessed treatment. The treatment was assessed from the making of the feed through the treatment process. The ORP was monitored with a Hanna Instruments edge® pH meter and HI36180 ORP probe. The electric conductivity was monitored with a Hanna Instruments edge® EC meter and HI763100 EC probe. The pH was measured with a Hanna Instruments edge® pH meter and HI11310 pH probe. The probes were sealed into a lid that fits on the container used for treatment. The lid could be sealed onto the water container with a flange. Bromine concentrations were measured with a Hanna Instruments Bromine Clicker after treatment.

The treatment section included all the equipment that formed part of the treatment process. 1000 mL cylindrical Perspex containers were used as water treatment containers. These containers could be sealed with a rubber ring, a lid, and a flange that was tightened with screws. The treatment container contained a magnet that was used for mixing on the magnetic stirrer. A Manson NSP-6016 power supply was used for ionisation. The power supply was connected to a Fluke 179 multi-meter that showed the average current that went through the ionisation system. A pipette was used to remove the required volume BCDMH solution from the BCDMH stock solution. A stopwatch was used to time the different treatment procedures.

The sterile microbiological section of the experimental apparatus was needed to do all the microbiological steps and monitoring of the treatment. In the microbiological section, all the diluting and plating had to be done as well as any transferral of bacteria-containing TSB. A Bunsen burner was used to keep the area sterile. Eppendorf tubes, pipette tips, Eppendorf tube holders, petri dishes with TSA, and glass flasks with TSB were the main equipment and consumables that formed part of the microbiological equipment.

Figure 40 is a labelled photo of the experimental setup. The monitors can be seen on the left. The power supply, multi-meter, electrodes, magnetic stirrer, and treatment container are in the middle. The sterile microbiological section is on the right adjacent to the Bunsen burner. Petri-dishes, a flask with a bacteria culture and an Eppendorf tube holder can be seen around the Bunsen burner.

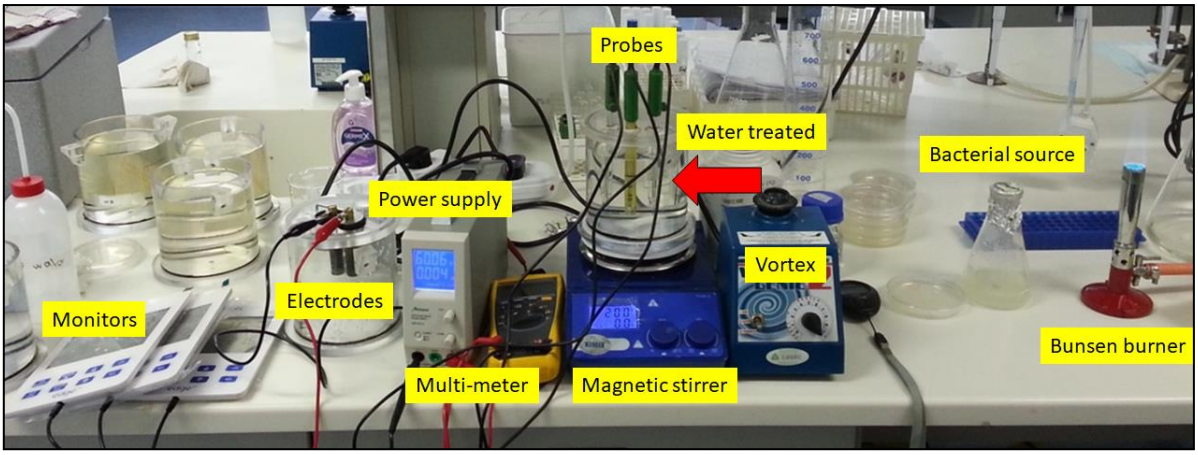

Figure 40: Experimental setup

3.6.3 Experimental procedure

The treatment procedure can be summarised by Figure 41. Equipment and consumables needed to be prepared before experiments were conducted. The feed preparation started a day before experimentation and ended with the final batch water to be treated. Continuous monitoring was implemented after the feed was prepared until after experimentation. The treatment itself consisted of ionisation and BCDMH addition. Finally, a sample was plated from the treated water to determine whether disinfection was successful.

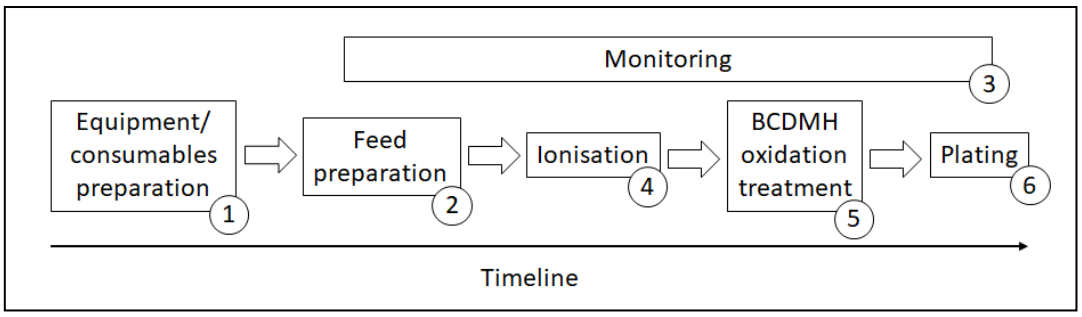

Figure 41: Treatment procedure

The first step for treatment to commence was the preparation of equipment and consumables. All the equipment needed, was sterilised before any experimentation could start. Equipment that was sterilised included glass beakers, measuring cylinders, treatment containers, pipettes, and magnets. Consumables that were sterilised were pipette tips, Eppendorf tubes and tap water. TSB, TSA, and saline solution were prepared and sterilised.

The feed preparation started a day before experimentation. The 100 mL TSB (3g/L), in a 250 ml Erlenmeyer flask, was inoculated with CT07 20 hours before treatment. The inoculated TSB was then incubated at 32°C until experimentation. After 20 hours, a 1 mL sample was removed from the

bacteria-containing TSB and the absorbance was measured. If the OD_{600} was above 0.600 then the bacteria culture was ready to be used for treatment. Another 1-mL sample was removed from the bacteria culture and diluted and plated to investigate the actual bacterial concentration. 891 mL sterilised tap water, measured with the measuring cylinder, was added to a treatment container followed by 9 mL bacteria-containing TSB, added with a pipette. The magnetic stirrer was switched on at 200 rpm. The treatment container would then contain 900 mL liquid, consisting of tap water with a bacterial concentration between 0.5 $x10^7$ and 2.0 $x10^7$ cfu/ml.

The monitoring process was started after the water was added to the treatment containers, before the bacteria containing TSB was added. The ORP, pH and EC probes were put in the water to be treated and the monitors were put on, logging the data every 5 seconds. The probes were kept in the water for a few minutes for measurements to become constant. The monitors were then switched off and the probes removed for the ionisation. Probes were rinsed with RO water before being inserted into the water and after removal from the water that was monitored.

The ionisation was started by putting the electrodes in the water and switching the power supply on. The stopwatch and multi-meter was started the moment the power supply was switched on. The stopwatch was used to determine when the power supply must be switched off, i.e. after the required ionisation time. The multi-meter measured the average current that passed through the system which was recorded after ionisation. The monitoring probes were put back in the water and the monitors switched on after ionisation.

The oxidation treatment required preparation that needed to take place the day before experimentation. BCDMH stock solution 1000 ppm was prepared by dissolving BCDMH powder in RO water 10 hours before the first experiment for the day. A pipette was used to add the required amount of BCDMH stock solution to the water that was treated to ensure the correct final concentration BCDMH in the water. The stopwatch was started with the addition of the BCDMH. After 5 minutes, two samples were removed with the automated pipette and plated on a petri dish. After plating, the monitors were switched off and the probes removed. The contaminated water was then treated with 90 mL bleach and left to stand for 30 minutes before it was discarded. The treatment container was rinsed thoroughly with RO water before being sterilised and prepared for experimentation a few hours later. The petri dishes were left for two days at room temperature before plate counts were taken.

After plating, the next treatment combination was started. A bacteria culture grown in TSB could be used for 2 hours as source for CT07 for batch feed preparation. Three bacteria cultures were grown per day at intervals of 2.5 hours which made it possible to do 8 hours of experiments in a day. Another controlled variable was temperature. All experiments were done at room temperature, 23°C ±2°C,

while water temperatures were kept between 20°C and 30°C. Small fluctuations in temperature were deemed insignificant because Landeen, Yahya et al. (1989) found that temperature did not influence the disinfection rate of ionisation-chlorination treatment (Landeen, Yahya et al. 1989).

The experiment combinations investigated can be broken into three categories. BCDMH was first investigated as disinfectant. Secondly, metal ions released through ionisation were investigated as disinfectant. Finally, the combination of metal ions and BCDMH was investigated as disinfectant. The first two sets of experiments investigated, had different contact times and served as the basis for the combined experiments. Experiments were randomised and repeated on different days to ensure repeatable results.

3.7 Analysis of experimentation

3.7.1 Requirements and choice of statistical analysis

The disinfection data consisted of binary data, i.e. disinfection was either successful or unsuccessful. The binary data had to be related to the ORP data and the treatment concentrations. ORP was monitored continuously with readings taken every 5 seconds. Due to the vast amount of data generated, statistical data analysis tool was therefore needed that could investigate the effect of two continuous independent variables on a binary dependent variable.

The analysis had to determine whether the ionisation disinfection and oxidation disinfection affected each other and whether the effect was constructive or destructive. Such an analysis would include whether there was interaction between the treatment processes and to what extent ionisation and oxidation effected the combined treatment efficiency. The analysed data had to be modified to create a model to predict disinfection. Such model could then be implemented to investigate the feasibility of the technology from other perspectives such as financial optimisation.

Logistic regression is the method most commonly used to analyse binary output data (Rodrıguez 2007, Kirkwood, Sterne 2005). Logistic regression is used to determine the weight different independent variables have on a binary output. These contributions are then summarised in a probability model. Logistic regression was therefore chosen as statistical tool to analyse the data and create models to represent the results. The logistic regression analysis was executed with the software package Statistica 64.

3.7.2 Logistic regression applied

Logistic regression, or logit, is a statistical analysis tool used to investigate and model the effects of different variables on a binary output. The independent variables investigated can be singular or

multiple, and can be binary, explanatory, or continuous in nature (Sperandei 2014). Logistic regression is an efficient tool to analyse several variables and their possible influence on a binary output. Logistic regression forms part of the tools implemented in machine learning. This section serves to give a brief explanation of logistic regression, the valuable statistical indicators, and how it was used to analyse the experimental data.

When working with binary data, the output can only be one of two possibilities. Mathematically these possibilities are defined as either "0" or "1". The purpose of the logit model is to find a relationship between the independent variables and the binary output variables. The model is built around investigating the probability (π) that a combination of independent variables will cause the binary output to be 1. The logit model is not necessarily linear, but dependent on the complexity of the relationship. The relationship between the independent variable and the probability for a specific binary output can be linear, quadratic, or of even a higher power. Equation 31 describes the logit equation to a cubic relationship. The equation can be rewritten to make probability the subject of the equation, Equation 32. For this research, references to the logistic regression model will refer to the logistic regression probability model (Kirkwood, Sterne 2005, Rodrıguez 2007, Sperandei 2014).

$$logit(\pi) = \log(\frac{\pi}{1-\pi}) = \beta_0 + \beta_1 x + \beta_{11} x^2 + \beta_{111} x^3 \quad \text{(Eq. 31)}$$

$$\pi = \frac{e^{\beta_0+\beta_1 x+\beta_{11} x^2+\beta_{111} x^3}}{1 + e^{\beta_0+\beta_1 x+\beta_{11} x^2+\beta_{111} x^3}} \quad \text{(Eq. 32)}$$

Where:

- π = probability for positive binary output, i.e. 1;
- β_0 = intercept;
- β_i = coefficient or weight of independent variable;
- x = independent variable.

When there are a variety of independent variables the model becomes much more complex. The logit model becomes more complex the more independent variables there are that are being investigated to influence the binary output. The interaction coefficient (β_{i2}) is the most valuable addition to the model when investigating combinations of different independent variables. For the purposes of the research only models with two independent variables will be discussed. The interaction coefficient serves as an indicator of whether the individual variables are exclusive or effect each other's influence on the binary output (Rodrıguez 2007, Sperandei 2014). A simple multi-variable logistic model with an interaction coefficient is described by Equation 33:

$$\pi = \frac{e^{\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_{12} x_1 x_2}}{1 + e^{\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_{12} x_1 x_2}} \quad \text{(Eq. 33)}$$

Where:

- π = probability for positive binary output, i.e. 1;
- β_0 = intercept;
- β_1 = coefficient or weight of variable x_1;
- β_2 = coefficient or weight of variable x_2;
- β_{i2} = interaction coefficient, or weight of interaction between variables x_1 and x_2;
- x_1 = amount of variable x_1;
- x_2 = amount of variable x_2;

Derived from the logistic regression, the odds ratio is often used to understand practically what the logit model means. The odds that an event will take place is the probability that an event occurs divided by the probability that it will not occur, Equation 34 (Sperandei 2014, Rodrıguez 2007).

$$Odds = \frac{\pi}{1 - \pi} \quad \text{(Eq. 34)}$$

The odds ratio is defined as the change in odds for an increase of one unit of the independent variable. When investigating a single independent variable in a linear logit model, the odds ratio can easily be calculated. The odds ratio for that independent variable is then e to the power of the variable coefficient, i.e. Equation 35. An odds ratio of 2.75 would mean that the odds for successful treatment would increase by 2.75 times for every increase by a single increment of the variable under investigation (Sperandei 2014, Rodrıguez 2007).

$$Odds\ ratio_1 = e^{\beta_1} \quad \text{(Eq. 35)}$$

There are two main approaches to determine the measure of fit of a logit model. The first approach looks at how well the model can be used to predict the binary output given a combination of independent variables. There are a variety of measures of predictive power, but the Cox-Snell R^2 is an acceptable test to use. The R^2 is a value between 0 and 1, the closer to 1 the more accurate are predictions (Allison 2014). The second approach looks at the goodness-of-fit of the data. This means how closely does the data fit the model and how can the model be improved to fit the data. There are different approaches to measure goodness-of-fit, but the Pearson Chi^2 is commonly used. The Chi^2 values are measured by p-values which determine whether the models are a good fit or not (Allison 2014).

It is necessary to practically link the logit model to the ionisation-oxidation disinfection experiments. The experimental binary output "successful" and "unsuccessful" disinfection was redefined as "1" and "0" respectively. The logit probability model represented the probability for "successful" disinfection. When the combination of ionisation and BCDMH was investigated, the more complex model that included the interaction coefficient β_{12} was investigated and compared to simpler models, i.e. β_0, β_1, β_2 and β_{12} were all investigated not to be zero. This was done because the interaction, if any, between the different disinfectants were to be investigated. The logistic model would also give a weight to each of the disinfectants and their effect on disinfection.

Statistica software gave a likelihood score for every possible logit model that combined the different measures of fitness. This score was used in combination with the Cox-Snell R^2, the Pearson Chi^2 and corresponding p-value, and Wald statistic to determine the most appropriate model to represent the relationship between disinfectants and disinfection. Cox-Snell R^2 values around 0.7 were defined as strong predictive value and correlation. It was decided that a model had to have a p-value < 0.001 to be significant. The Wald statistic is often used to investigate the null hypothesis for the coefficients (β_j and β_{ij}). The null hypothesis (H_0) was that all the coefficients were zero, i.e. $\beta_j = 0$. A p-value < 0.1 was chosen to reject the null hypothesis for every coefficient investigated (Rodrıguez 2007, Sperandei 2014).

The logistic regression analysis has its own limitations. Logistic regression is slightly different from other regression models and is, therefore, interpreted differently. Wald statistical indicators are overly conservative when a larger ratio of variables is analysed. The likelihood score can be manipulated by excluding the extreme values that do not influence the actual model. When this is done, the model coefficients remain basically the same, but the likelihood can be increased. The likelihood score works well to decide which of the variables to include in the model. The Cox-Snell R^2 value is more difficult to interpret than R^2 values used in other regression analysis, and are generally lower. The difference between model p-values and coefficient p-values can further complicate the analysis. The choice to use a p-value of 0.10 for the coefficients decrease the general model strength.

4.Results and discussion

The first section, *4.1 Introduction*, explains the chapter and the main content. Section *4.2 Disinfection treatment* discusses BCDMH disinfection results, metal ion disinfection results, and combined disinfection results. Section *4.3 Assessing disinfection treatment* explains the potential of ORP as an ionisation-oxidation disinfectant control.

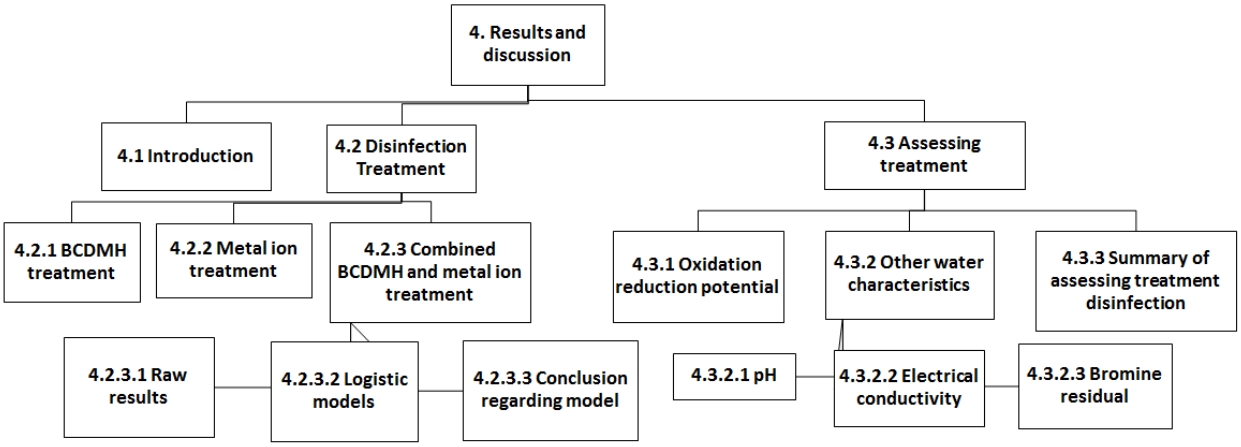

Figure 42: Results and discussion structure

4.1 Introduction

The purpose of this chapter is to convey to the reader the results from the experimentation, the analysis thereof, and the interpretations of the results. Generally, the results mentioned and interpreted are related to the objectives of the research and function as stepping stones to reach the objectives. The first and third objectives are discussed in this chapter, while the second objective is discussed in the next chapter, *5. Feasibility of ionisation-oxidation disinfection*. The first and third stated objectives of the research were:

4) To identify the contribution, if any, of metallic ions on the disinfection ability of BCDMH.
3) To evaluate ORP as an indicator of disinfection efficacy for a disinfection process that combine metal ions with BCDMH.

These two objectives were investigated simultaneously, but the results have been broken down into two different sections. The first section discusses the disinfection treatment experiments, and the results are given in the following sequence. First BCDMH disinfection results are given and discussed as a singular treatment process. Then metal ion treatment and its application as a point-of-use water disinfectant is discussed. Finally, the combined disinfection of metal ions with BCDMH is discussed.

The second section discusses the assessment of disinfection, and focuses on ORP and the different ways ORP can be used to assess disinfection. There is also a short section on other characteristics of the disinfection process that include changes in pH, EC, and bromine residual that were monitored to further understand changes in ORP and to correlate ORP to disinfection success.

4.2 Disinfection treatment

4.2.1 BCDMH treatment

To develop a practical understanding of BCDMH as disinfectant to treat CT07 in batch conditions, a sequential experimental process was followed. First a preliminary scan was done to investigate BCDMH as disinfectant for a 5-miute contact time. The results were used to do a more comprehensive investigation into the concentration-contact time (CT) relationship of BCDMH. The CT experiments led to further experiments on BCDMH disinfection for a 5-minute contact time. These results were analysed with logistic regression and a model that connected the probability for successful disinfection with BCDMH concentration was developed.

The preliminary experimentation served to gauge the effectiveness of BCDMH as disinfectant, the repeatability of treatment results, and at which concentrations BCDMH could be used as treatment. A variety of BCDMH concentrations were investigated from 0 ppm BCDMH, the control, to 100 ppm BCDMH. The experiments were done in triplicate and investigated a 5-minute contact time. Figure 43 graphically displays the results.

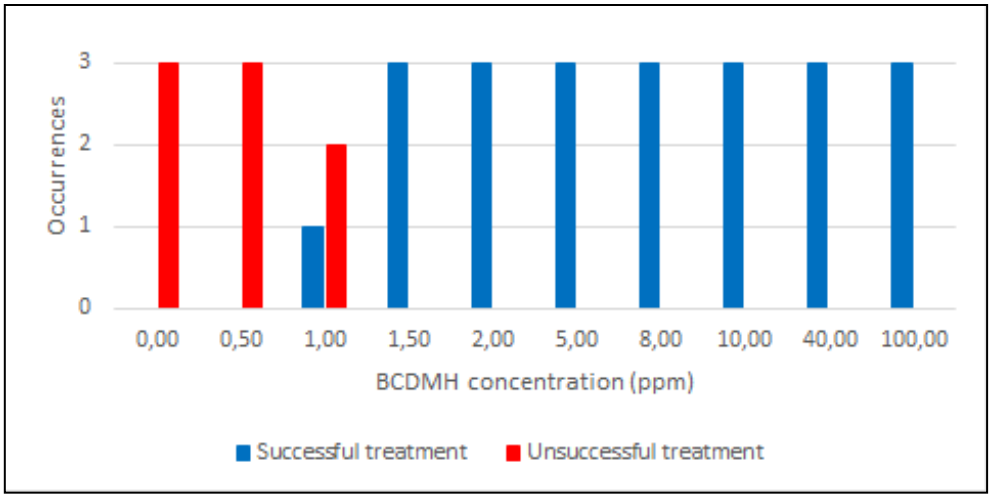

Figure 43: Disinfection effectiveness of different BCDMH concentrations

BCDMH concentrations of 1.5 ppm and above showed repeated success in disinfection, while concentrations of 0.5 ppm and below showed repeated unsuccessful disinfection. A concentration of 1 ppm was sometimes successful in treating the batch feed, but was more often unsuccessful. BCDMH

concentrations between 0.5 and 1.5 ppm needed to be investigated for a clearer understanding of BCDMH disinfection and to identify where the treatment cut-off-point was.

The CT experiments were decided on considering the results of the preliminary scan. BCDMH concentrations of 0.8, 1.0, 1.2, 1.4, and 1.6 ppm were investigated as well as a control of 0.0 ppm. Samples were taken and plated after 0, 5, 15, 30, and 45 minutes. Figure 44 is a scatter plot of the BCDMH concentration and contact time for successful and unsuccessful disinfection.

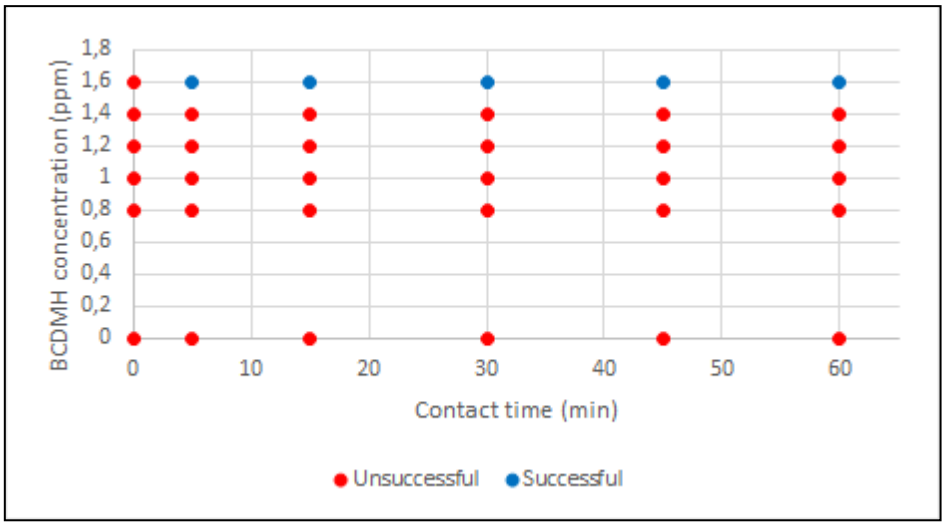

Figure 44: Disinfection success for different BCDMH concentrations and different contact times

A BCDMH concentration of 1.6 ppm was the only treatment that was successful as disinfectant, and already after a 5-minute contact time. Treatment of 1.2 and 1.4 ppm BCDMH both decreased bacterial concentrations dramatically, but did not decrease them to below detection limits. The 1.2 ppm BCDMH decreased the bacterial concentration to less than 250 cfu/ml within a 30-minute contact time, where after bacterial concentrations remained constant. Similarly, the 1.4 ppm BCDMH treatment decreased the bacterial concentration to less than 250 cfu/ml within a 15-minute contact time, with bacterial concentrations remaining constant thereafter. The bacterial concentrations that stopped decreasing is probably due to no more free chlorine or bromine available for disinfection.

From the CT experiments, it was observed that the bacterial concentrations in samples did not decrease further after 30 minutes contact time for BCDMH concentrations between 1.2 and 1.6 ppm. BCDMH, therefore, seemed to be most effective as biocide for the first 30 minutes of contact time. These results were in line with literature, which mentioned that halogen based oxidising disinfectants require a short contact time for disinfection (Takahashi, Kirihara et al. 2005, Howarth 2010, Kim, Anderson et al. 2002). It was clear that BCDMH treatment could be efficient for short contact time treatments such as 5 minutes. All the future BCDMH related experiments were done for a contact time of 5-minutes to represent point-of-use water treatment. The CT experiments did not give

sufficient data to develop a full CT relationship, and since it was not part of the scope of the research, it was not further investigated.

The efficiency of the BCDMH treatment differed slightly throughout the initial experiments. The required BCDMH concentration to disinfect the feed varied from 1.0 ppm to 1.6 ppm. Repeated 5-minute experiments were needed to do a logistic regression analysis and to develop a probability model for successful disinfection. A variety of BCDMH treatments were done at different concentrations with repetitions to investigate the relationship between BCDMH concentration and disinfection success for a 5-minute contact time. Logistic regression was used to create a probability model that gives the probability for successful disinfection for any BCDMH concentration. Equation 36 describes the model mathematically.

$$\pi = \frac{e^{-9.449+7.158x}}{1 + e^{-9.449+7.158x}} \qquad \text{(Eq. 36)}$$

Where:

- π = the probability for successful disinfection,
- x = the BCDMH concentration (ppm).

The model is significant with a p-value <0.001 and a Cox-Snell R^2 of 0.601. The β_0 is -9.449 and β_1 is 7.158, with Wald statistic p-values equal to 0.001 and 0.003 respectively. Both p-values are <0.1 and therefore significant.

The logit model can be discussed further and represented visually. The logit analyses gave BCDMH concentration an odds ratio of 1284.780. This means that with every increase of 0.1 ppm BCDMH treatment, the odds for successful disinfection increases with 104.59%. Figure 45 is a visual representation of the model.

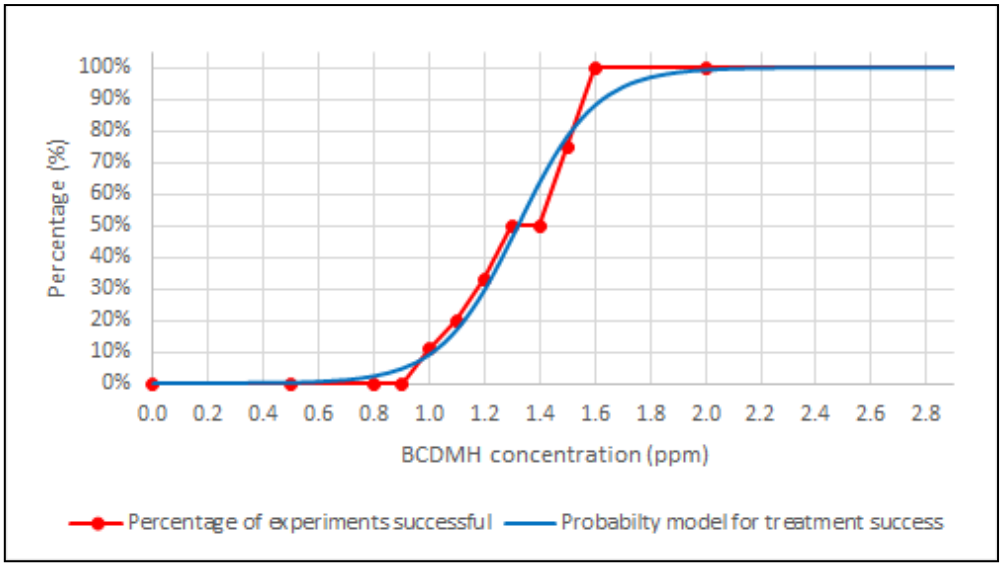

Figure 45: Experimental data for BCDMH treatment compared to probability model for successful disinfection

The blue line representing the model defined by Equation 36 and the red line the percentage of experiments that were successful for different BCDMH concentrations. From the graph, it is obvious that experimental data for treatment success correlates with the created model that can be used to predict treatment success. This would be expected from a Cox-Snell R^2 of 0.601. The model can be used to show that a BCDMH treatment of 1.320 ppm has a 50% probability of being successful. A treatment of 1.627 ppm BCDMH, on the other-hand, has a 90% probability of being successful.

From all the experiments with BCDMH as disinfectant, it was possible to say that BCDMH is effective as disinfectant at concentrations above 1.6 ppm for a 5-minute contact time. The logit model predicts a 90% probability for successful disinfection for BCDMH concentrations of 1.627 ppm. Therefore ionisation-oxidation treatment combinations needed to be investigated for BCDMH concentrations below 1.6 ppm and for ionisation below environmental safety standards. Experiments in the above-mentioned regime would improve understanding of the effect of ionisation, i.e. metal ions, on BCDMH disinfection.

4.2.2 Metal ion treatment

According to literature, metal ions require a few hours, or even days, contact time to ensure disinfection (Fewtrell 2014, Jung, Koo et al. 2008, Huang, Shih et al. 2008). A preliminary investigation into disinfection with copper, silver, and zinc ionisation found similar results. The investigation showed that for ionisation that released 6.060 coulomb electrons per litre, 60 minutes was necessary for successful disinfection. For ionisation that released 13.422 coulomb electrons per litre, 30 minutes were required for disinfection.

A second set of experiments were designed to investigate the CT for ionisation that would release an allowable amount of metal ions. The feed was treated with 5 different ionisation concentrations and a control experiment was done without treatment to ensure the bacteria does not die due to a lack of nutrients. Samples were plated after 0, 5, 15, 30, 45, 60, 75 and 90 minutes after treatment. Only ionisation of 6.421 and 9.373 coulomb electrons per litre showed successful disinfection and only after a contact time of 90 minutes. Figure 46 shows the ionisation experimental data for the preliminary and CT investigations, as well as the precautionary ionisation limit.

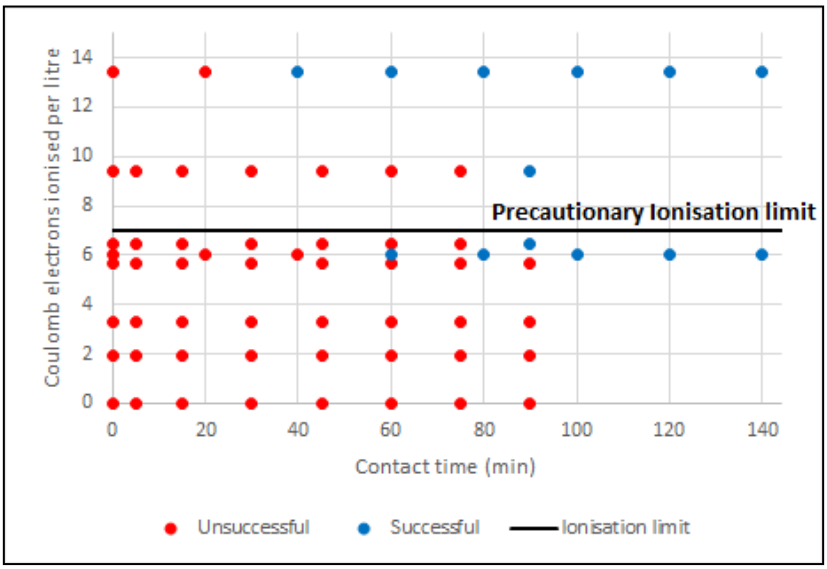

Figure 46: Coulomb electrons released and contact time compared with treatment success

From Figure 46 several deductions could be made. For below the health limit on ionisation, a contact time of 60 minutes seem necessary to attain successful disinfection. An increase in ionisation, i.e. an increase in metal ion treatment, did not decrease the contact time dramatically. The rate of disinfection was not greatly affected by the degree of ionisation. The black line represents the ionisation limit calculated from the SANS limit on copper concentrations in drinking water of 2.0 ppm. Ionisation that removes 7 coulomb electrons per litre water treated results in approximately 2.0 ppm copper concentration (SABS 2015). From the earlier ionisation experimentation, it can be deduced that an increase in ionisation would not necessarily improve disinfection because of the metals precipitating. A comprehensive investigation into the CT relationship for ionisation treatment may be interesting, although it does not fall within the scope. The information obtained was sufficient to investigate the combined treatment.

When comparing BCDMH and metal ion treatment, BCDMH treatment can be implemented and within minutes the water can be free of pathogens. When considering point-of-use water treatment, ideally water treatment should be quick and not time consuming. Metal ions, however, require longer contact

times and did not successfully disinfect any feed under 30 minutes. Metal ionisation is, therefore, less ideal for point-of-use water treatment.

Since the objective of the research was to investigate the contribution of metal ions to BCDMH disinfection, it made sense to investigate the 5-minute contact time for the combined technology. Ionisation would not result in a successful disinfection in 5 minutes. BCDMH would cause successful disinfection at concentrations of about 1.6 ppm for 5 minutes contact time. Any improvement in a combined ionisation-oxidation process would give feedback on ionisation improving the oxidising agent's disinfecting ability.

4.2.3 Combined BCDMH and metal ion treatment

4.2.3.1 Raw results

The core of the first objective of the research is focused on the combined disinfection with BCDMH and metal ions. The first experiments determined that BCDMH can be implemented as disinfectant for the feed under investigation at concentrations of approximately 1.6 ppm for a 5-minute contact time. The experiments also determined that metal ions will not sufficiently disinfect within a 5-minute contact time. Combined treatment that showed improved disinfecting capabilities over the independent BCDMH treatment would support the hypothesis that there is interaction between the different disinfecting mechanisms.

The experimental combinations, or experimental regime, were determined by a trial and error method. From the BCDMH treatment experiments, it was decided to investigate BCDMH concentrations between 0.75 and 1.60 ppm. Metal concentrations investigated were to be kept below the environmental recommendations, which was equivalent to 7 coulomb electrons ionised per litre. The ionisation investigated was, however, chosen to vary from 0 to 10 coulomb electrons ionised per litre to get a broader treatment regime to investigate. The treatment combinations were designed in subsets, using the results of an initial subset of experiments to design a successive set of experiments. Half of a successive set of experiments always overlapped with the previous set of experiments.

The experimental protocol was followed to combine ionisation treatment with BCDMH treatment. The separate sets of experiments can be seen Appendix B. Figure 47 represents the results of the experiments within the identified regime.

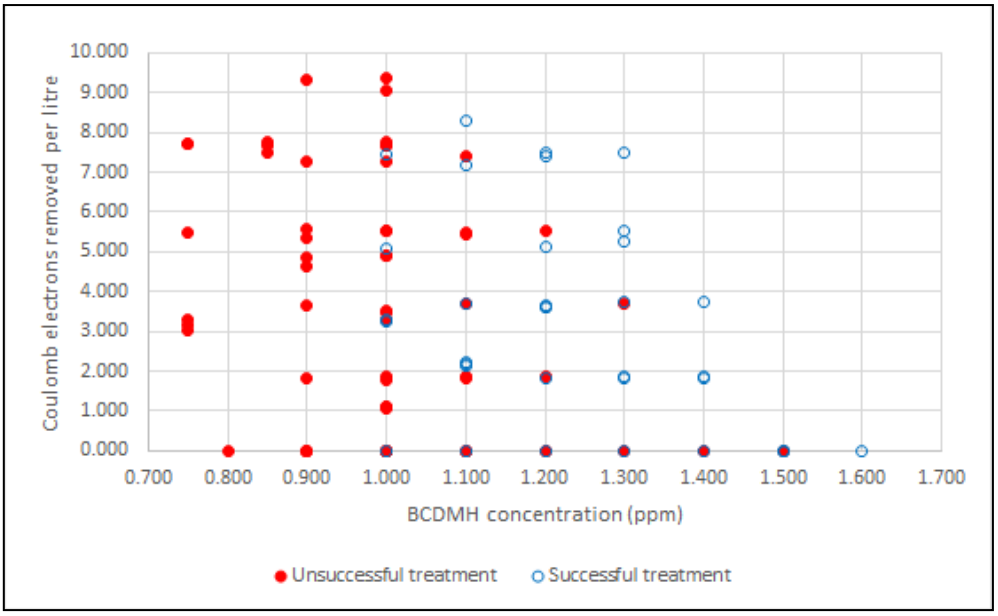

Figure 47: Treatment effect on bacteria for all the experiments

Successful disinfection is indicated by blue circles while unsuccessful disinfection is indicated by red dots. The figure does not clearly distinguish between different experiments and therefore repeated experiments cannot be seen, as the red dots or blue circles overlap. There is, however, a clear distinction between combinations that are more prone to attain successful disinfection and those combinations which do not. From the data points on Figure 47, BCDMH concentrations below 1.0 ppm never attained successful disinfection. On the other hand, combinations of high BCDMH concentrations with high ionisation show mostly successful disinfection.

The experimental data was grouped to the nearest whole coulomb electron ionised per litre. The grouped experimental results is represented by Table 4.

Table 4: Experimental results for combined ionisation and BCDMH treatment

		Disinfection success																			
		0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1
Coulomb electrons ionised per litre	9	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0
	8	2	0	0	0	2	0	0	0	3	0	0	1	0	1	0	0	0	0	0	0
	7	0	0	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0	0	0	0
	6	0	0	0	0	0	0	1	0	2	0	0	0	1	0	0	1	0	0	0	0
	5	1	0	0	0	0	0	3	0	2	1	2	0	0	1	0	1	0	0	0	0
	4	0	0	0	0	0	0	1	0	1	0	1	1	0	3	1	1	0	1	0	0
	3	3	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	1	0	2	0	2	3	1	1	0	2	0	2	0	0
	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	1	0	0	0	4	0	8	1	4	1	2	1	1	1	1	1	1	3
		0.75		0.8		0.85		0.9		1.0		1.1		1.2		1.3		1.4		1.5	
		BCDMH concentration (ppm)																			

The data in the table helps understand the spread of the experiments. As from Figure 47, it can be seen that no treatment combination disinfected successfully for BCDMH concentrations below 1.0 ppm. The most experiments were done for the BCDMH concentrations between 0.9 ppm and 1.3 ppm with ionisation that varied from 0 to 8 coulomb electrons ionised. The table represents 101 of the 131 experiments done.

4.2.3.2 Logistic regression models

The binary data was analysed using logistic regression as discussed in section *3.7.2 Logistic regression applied*. The model investigated, included a disinfecting effect by the BCDMH concentration, coulomb electrons released and the combined interaction of the two different disinfectants. I.e. β_0, β_1, β_2 and β_{12} were all investigated not to be zero. The analysis, therefore, considered all three factors and the likelihood that they influence the success of treatment. All the experimental data was used for the logistic regression. Table 5 contains the different combinations the analyses identified, and their likelihood scores as well as their p-values.

Table 5: Logit models for disinfection with likelihood scores

All data sets	Disinfection - Model building results					
	Distribution : BINOMIAL					
	Link function: LOGIT Modeled probability that Disinfection = 1					
	Var. 1	Var. 2	Var. 3	Degr. of Freedom	Likelihd Score	p
1	BCDMH concentration	Coulomb electrons	Interaction	3	23.961	0.000025
2	Coulomb electrons	Interaction		2	15.183	0.000505
3	BCDMH concentration	Interaction		2	12.733	0.001718
4	BCDMH concentration	Coulomb electrons		2	10.962	0.004165
5	BCDMH concentration			1	10.906	0.000959
6	Coulomb electrons			1	4.111	0.042599
7	Interaction			1	0.175	0.675934

The model that included all three factors had the highest likelihood score of 38,946 with a p-value <0.001 and was therefore chosen as model to be investigated. The 2 factor models all had p-values <0.001 but their likelihood scores were much lower than the 3-factor model. The model with only BCDMH concentration as a factor, had the highest likelihood score of all the single variable models.

The logistic regression analyses produced the probability model that is described by Equation 37.

$$\pi = \frac{e^{-8.529+6.654x_1-1.782x_2+1.816x_1x_2}}{1+e^{-8.529+6.654x_1-1.782x_2+1.816x_1x_2}} \quad \text{(Eq. 37)}$$

Where:

- π = the probability for successful disinfection,
- x_1 = the BCDMH concentration (ppm),
- x_2 = the intensity of ionisation (Coulombs electrons/L).

The model is significant with a p-value <0.001 and has a relatively strong correlation with a Cox- Snell R^2 of 0.516. β_0 is equal to -8.534 and is significant with a p-value of <0.001. β_1 is also significant being equal to 6.658 with a p-value of 0.0013. β_1 is related to the effect a change in BCDMH concentration will have on probability for disinfection. β_2 is equal to -1.782, but is slightly less significant than β_0 and β_1, with a p-value of 0.057, but still significant because it is <0.1. β_2. β_2 is related to the effect a change in ionisation intensity will have on probability for disinfection. β_{12} is equal to 1.816 and significant with a p-value of 0.036. β_{12} is related to how the separate disinfectants influence each other and the probability for disinfection.

The interpretations of the beta values are usually assessed by looking at the change in odds, or odds ratio. However, due to the interaction between BCDMH and metal ion treatment, the odds ratio is not constant. The significant interaction coefficient, β_{12}, causes the odds ratio to be dependent on both variables and needs further explanation. An increase in 0.1 ppm BCDMH concentration can cause an increase in the odds for successful disinfection by 94% if no ionisation is done. If 7 coulomb electrons are ionised per litre, an increase in 0.1 ppm BCDMH concentration will increase the odds by 593.68%. From the negative β_2 it would be expected that an increase in ionisation would decrease the odds for successful disinfection, but the interaction coefficient causes the opposite effect. A change in ionisation intensity that releases an additional 1 coulomb electron per litre will cause the odds for successful disinfection to increase by between 24.08% and 156.53% for BCDMH concentrations between 1.1 and 1.5 ppm. An increase in 1 coulomb electron ionised per litre and an increase in 0.1 ppm BCDMH will increase the odds ratio by 43.79%.

Generally, visual interpretations of models make them easier to understand. The model is visually represented by the contour lines on Figure 48.

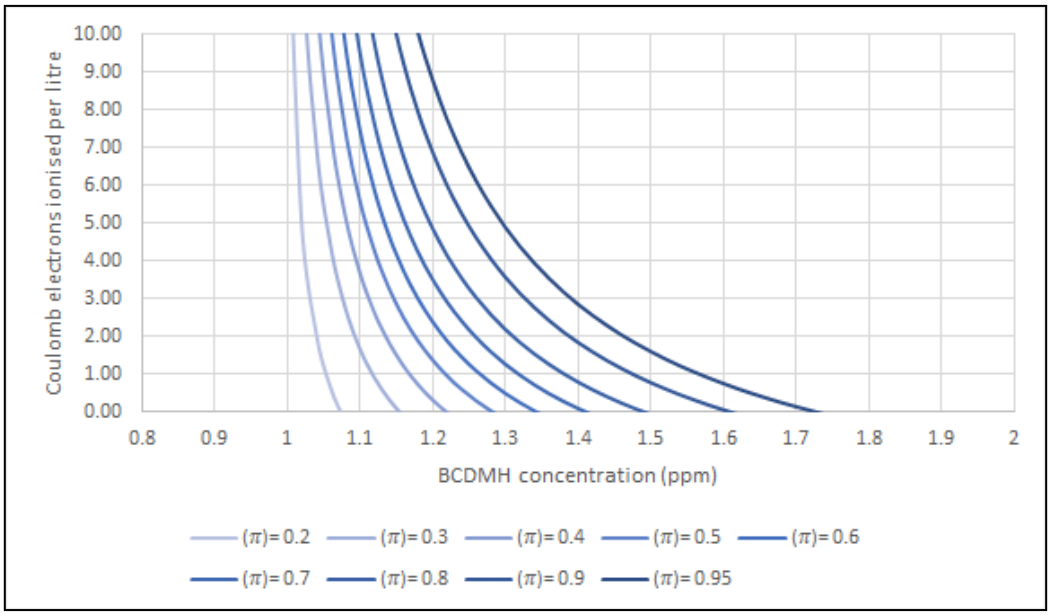

Figure 48: Probability for successful treatment model 1

The darker lines show a higher probability for successful disinfection for combinations of treatment for BCDMH and ionisation. From right to left are the higher probabilities to lower probabilities for successful disinfection. The contour lines are positioned closely at the top and further apart at the bottom. This means the change in probability happens with small increases in BCDMH at high ionisation rates but with large increases in BCDMH at low ionisation rates. For the model, the interaction between BCDMH concentration and ionisation has a significant effect on disinfection success, as the interaction coefficient is significant. The shape of the 0.9 probability curve gives a visual understanding to the interaction. The model is also similar to the BCDMH treatment model developed in section *4.2.1 BCDMH treatment*. This model requires 1.61 ppm BCDMH for only BCDMH treatment for a 90% probability for successful treatment, compared to the BCDMH model that required 1.63 ppm BCDMH.

The ionisation only disinfection experimentations showed that metal ions do not disinfect for short contact time treatment. Model 1, developed in Equation 37, had a β_2 with a p-value slightly >0.05, but below the defined significant required value of 0.1. If an analysis would require that all the coefficients had to be more significant, model 1 would be rejected. A second, alternative, model was investigated that could be compared with model 1. The third model from the different logit models in Table 5 was chosen. According to Table 5 the model had a p-value of 0.0017, which is >0.001 which was required for a significant model. When some of the extreme values were removed from the analysis, the p-

value dropped to <0.001 while the coefficients did not change significantly. The data points considered extreme values were any treatment combination with 0 ppm BCDMH or more than 8 ppm BCDMH. The second model and fourth model in Table 1 both had coefficients that were not significant and fitted the data poorly.

The model that considers the effect of BCDMH concentration, as well as the interaction effect of BCDMH and ionisation, was therefore investigated. Another reason for the specific choice, was the results of the earlier separate disinfection investigations. It is known that BCDMH will cause disinfection when used alone as disinfectant, and an increase in BCDMH concentration will increase the probability for successful disinfection. Metal ionisation, on the other hand, will not cause disinfection on its own at low or high intensity for a 5-minute contact time. Therefore, it makes sense to assume that $\beta_2 = 0$, as any increase or decrease in only ionisation treatment will not result in successful disinfection for a 5-minute contact time.

The second model investigated differ from the first model primarily in that the individual disinfection effect of the ionisation is ignored, but the models form different curves. The second model is significant, with a p-value <0.001, and correlated strongly with a Cox-Snell R^2 of 0.4366. The correlation is weaker, and the p-value is slightly larger than the first model. The second probability model can be described by Equation 38.

$$\pi = \frac{e^{-12.069+9.740x_1+0.182x_1x_2}}{1+e^{-12.069+9.740x_1+0.182x_1x_2}} \quad \text{(Eq. 38)}$$

Where:

- π = the probability for successful disinfection,
- x_1 = the BCDMH concentration (ppm),
- x_2 = the intensity of ionisation (Coulombs electrons/L).

β_0 is equal to -12.069 and significant with a p-value <0.001. β_1 is equal to 9.740 which means an increase of at least 164.86% in odds for successful disinfection for an increase in 0.1 ppm BCDMH concentration with a significant p-value <0.001. If the ionisation is 7 coulomb electrons ionised per litre, then an increase in 0.1 ppm BCDMH treatment will increase the odds for successful treatment by 200.87%. β_{12} is equal to 0.182 with a significant p-value of 0.046. All the coefficient p-values are <0.05. The β_{12} is relatively small and means the combined effect of an increase in 0.1 ppm BCDMH concentration and an increase in 1 coulomb electrons removed through ionisation cause an increase of 3.71% in the odds ratio. An increase of 1 coulomb electron ionised per litre can increase the odds

for successful disinfection by between 22.18% and 31.41% for BCDMH concentrations between 1.1 and 1.5 ppm.

Figure 49 visually represents the second model.

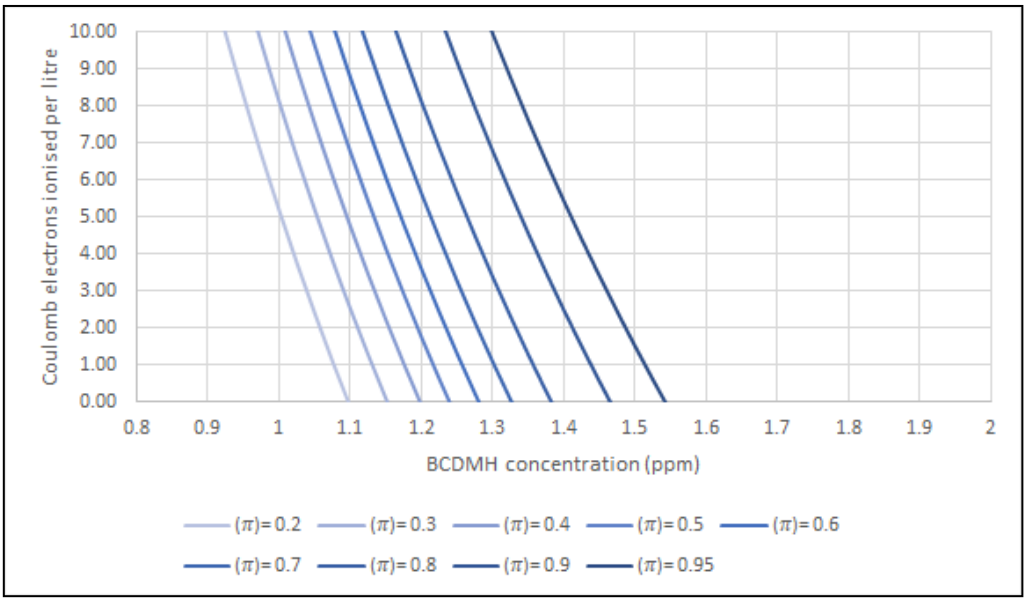

Figure 49: Probability for successful treatment model 2

The contour lines represent the different probabilities for successful treatment for different treatment combinations. The probability lines are very close to being straight lines. If the combined treatment had only an additional effect and no interaction, the probability contours would have been straight lines. If only BCDMH concentration would influence disinfection, then the probability contours would be vertical lines. Visually, the metal ionisation improves disinfection. However, the odds ratio for the interaction is very small compared to the odds ratio for the BCDMH concentration. This model supports the hypothesis that there is a significant interaction between ionisation and oxidation. However, the interaction is smaller than for the first model and, therefore does not influence BCDMH concentrations as dramatically.

It is important to be aware of the differences between model 1 and model 2. Figure 50 represents the differences, between model 1 and model 2, in BCDMH required for different intensities of ionisation to achieve specific probabilities for successful treatment.

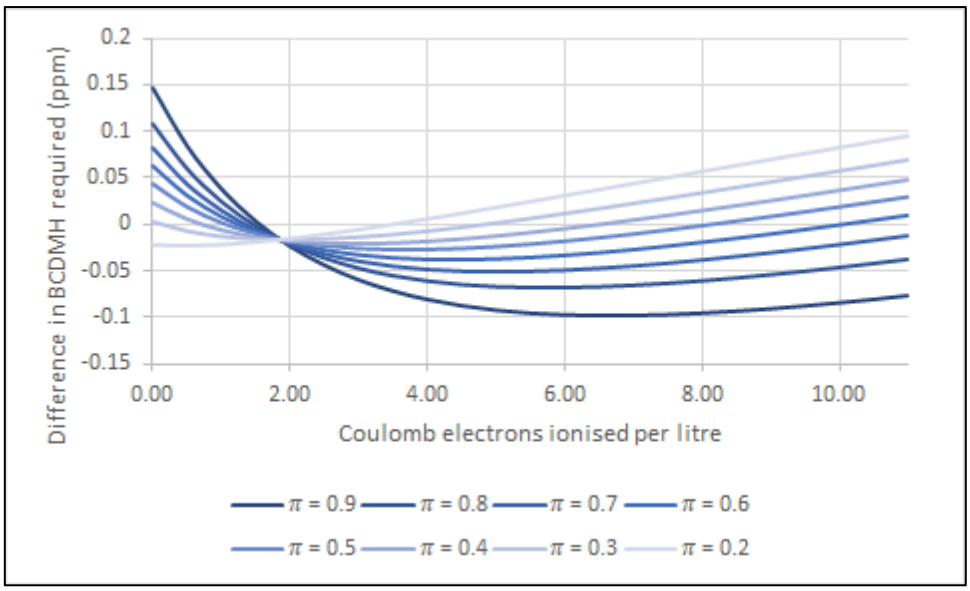

Figure 50: Difference in BCDMH concentrations required for different ionisation concentrations for model 1 vs model 2

The darker blue lines represent a larger probability for successful treatment and the lighter lines represent lower probabilities. For different required probabilities the models vary drastically. For a target probability of 0.9, model 1 requires 0.15 ppm BCDMH more than model 2 for no ionisation, but requires approximately 0.1 ppm BCDMH less when combined with 7 coulomb electrons ionised per litre. Model 1 and model 2 differ less for a target probability of 0.5. The absolute difference in BCDMH concentration is then most of the time between 0.02 and 0.03 ppm.

Model 1 and model 2 both have advantages and disadvantages. The likelihood score of model 1 is 23.961 compared to the 12.733 of model 2 when analysing all the experimental data. When considering the same data set, model 1 has a higher Chi-square likelihood ratio, larger likelihood score, smaller p-value, and larger Cox-Snell R^2, which all contribute to make model 1 more attractive. Model 2, on the other hand, has more significant beta values, with beta p-values <0.05 compared to model 1 with beta p-values <0.1. Model 1 has a larger and more significant interaction coefficient which means it effects the probability for successful disinfection more and is closer related to the experimental data.

The BCDMH only treatment, investigated initially, had a strong correlation to the corresponding model developed. Figure 51 compares the three probability models for only BCDMH treatment.

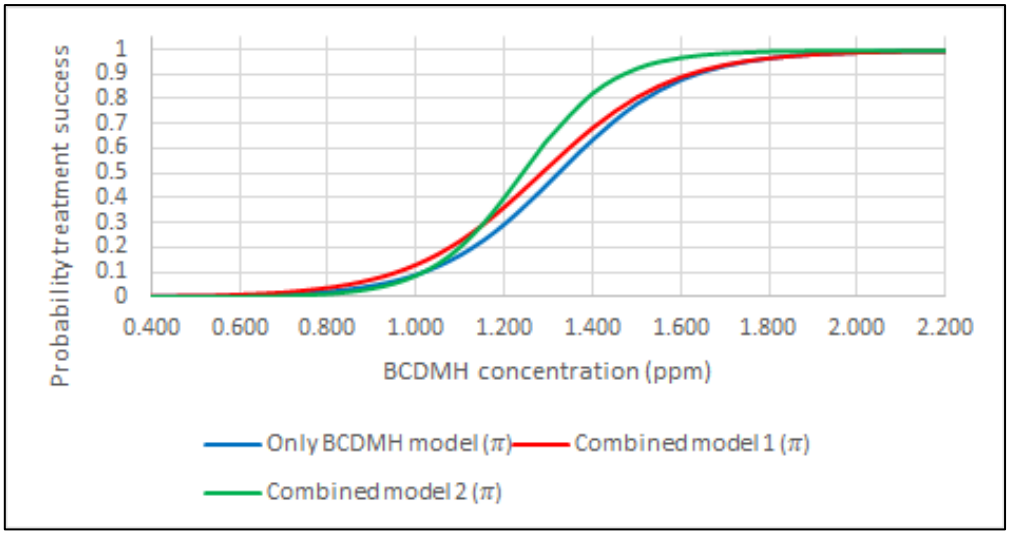

Figure 51: Probability for treatment success for only BCDMH treatment comparing model 1, model 2, and the BCDMH model

Model 1 is more similar to the BCDMH model than the model 2 from BCDMH concentrations above 1.2 ppm. At concentrations above 1.5 ppm BCDMH, the BCDMH model and model 1 are basically identical. At low concentrations, model 2 is like the BCDMH model. When it comes to disinfection, it is more important to have models that are accurate at the high probability of treatment success because that is where treatment will be implemented.

Figure 52 shows the experimental data compared with the two models for a probability of 0.9 for successful treatment.

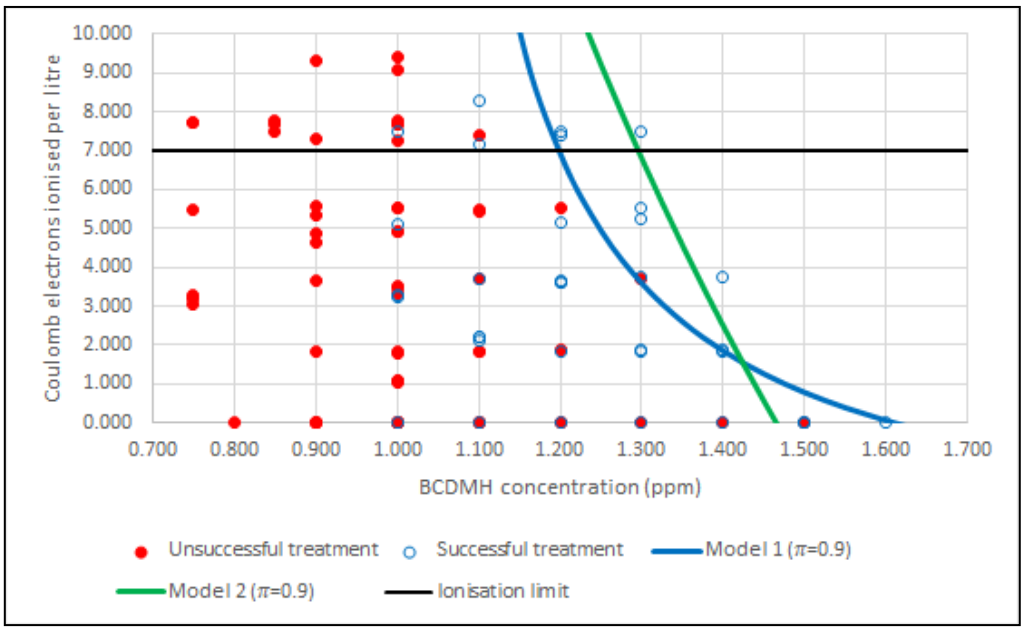

Figure 52: Treatment effect on bacteria with logit models for P = 0.9

Visually model 1 seems to correlate more with the experimental data which is supported by the calculated correlation indicator, the Cox-Snell R^2. Model 2 seems to be a simplified model. Model 1 shows a decrease in 25.67% BCDMH concentration required for a 90% probability for successful treatment by increasing ionisation from 0 coulomb electrons ionised to 7 coulomb electrons ionised per litre. Model 2 only shows an 11.57% decrease in BCDMH concentration required for a 90% probability for successful treatment for the same change in ionisation. Model 1, therefore, shows more than 100% higher efficiency than model 2 for the range under investigation. On the other hand, for a targeted 50% probability for successful treatment, the models look very similar and yield similar benefits for the combined treatment.

4.2.3.3 Conclusions regarding models

To simplify the feasibility investigations, one model had to be chosen to be used for the rest of the research. It is necessary to summarise the two models before explaining the model that was chosen for the feasibility investigation. Model 1 is more complex than model 2, containing an additional coefficient. Both models contain interaction coefficients that are significant and support the synergistic interaction between ionisation and BCDMH disinfection. When considering most statistical indicators, model 1 is a better fit as model, except for the ionisation coefficient that has a p-value of 0.057. Model 2 is more simplistic and easier to use than model 1, but the overall model does not correlate with the data. From a visual perspective, model 1 fits the experimental data better. Model 2 is more conservative, as the decrease in BCDMH due to ionisation is less than half proposed by model 1.

Taking all the advantages and disadvantages into account, model 1 was the model chosen to be used to represent the disinfection probability. Equation 39 describes the probability for successful disinfection with x_1 being the BCDMH concentration and x_2 the coulomb electrons removed from the anode through ionisation per litre.

$$P = \frac{e^{-8.534+6.658x_1-1.782x_2+1.816x_1x_2}}{1+e^{-8.534+6.658x_1-1.782x_2+1.816x_1x_2}} \quad \text{(Eq. 39)}$$

Where:

- π = the probability for successful disinfection,
- x_1 = the BCDMH concentration (ppm),
- x_2 = the intensity of ionisation (Coulombs electrons/L).

The probability model is visually displayed by the 3D model in Figure 53.

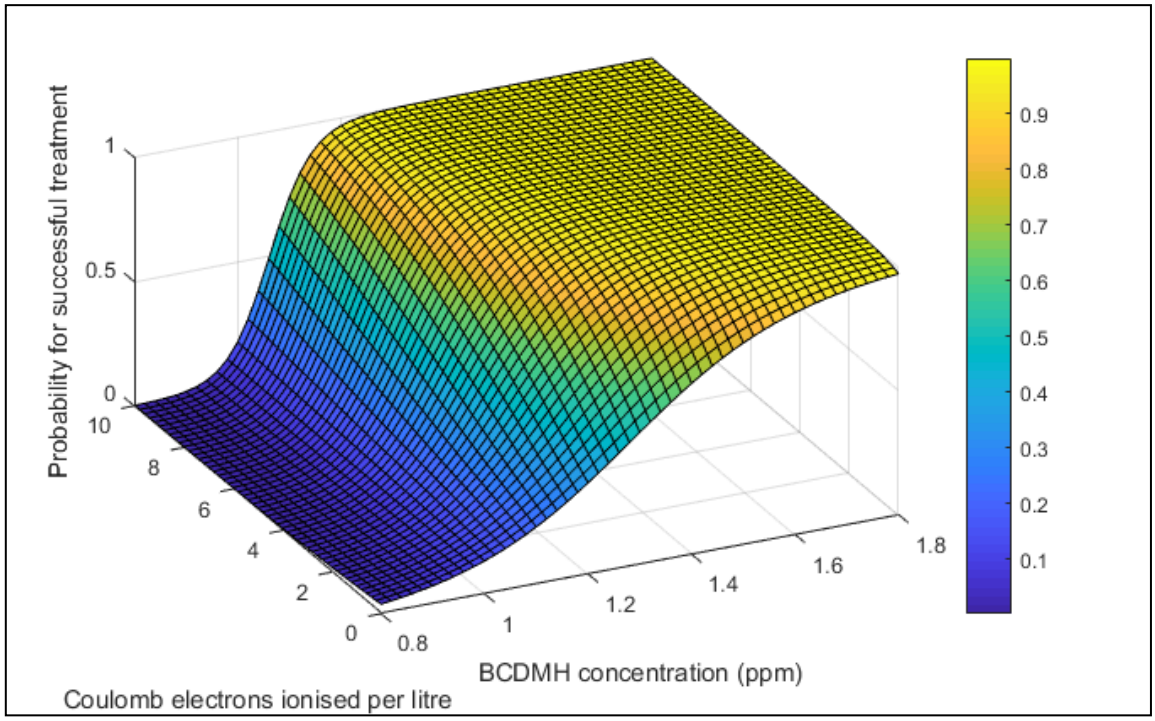

Figure 53: 3D probability model for successful disinfection

The interaction coefficient is significant and proofs the metal ions play a role in disinfection. Ionisation strengthens the disinfection capabilities of the oxidising agent BCDMH. Practically, when requiring a 95% probability for successful treatment, the BCDMH concentrations can be decreased by 29.54% when it is combined with the maximum allowable ionisation treatment.

4.3 Assessing disinfection treatment

4.3.1 Oxidation reduction potential (ORP)

Some general trends of ORP values were identified and are discussed below. The investigations to correlate ORP to disinfection and use it as control for ionisation-oxidation treatment, is also discussed below.

Figure 54 shows the monitored ORP for one of the ionisation-oxidation disinfection treatment experiments. The ORP graphs for all the experiments can be seen in the Appendix C. The trends that can be seen in Figure 54 typical for such a treatment process and a few general observations were made. The initial ORP of the tap water generally took several minutes to stabilise, and was often between 200 and 300 mV. The addition of the TSB with bacteria, which has an ORP of about 300 mV, often caused the ORP to increase initially and then to decrease again slightly. The ionisation caused a decrease in ORP, but the BCDMH was added without giving sufficient time for the ORP to stabilise.

The addition of BCDMH caused an immediate increase in ORP, thereafter the ORP would either continue to increase, remain constant, or start to decrease again.

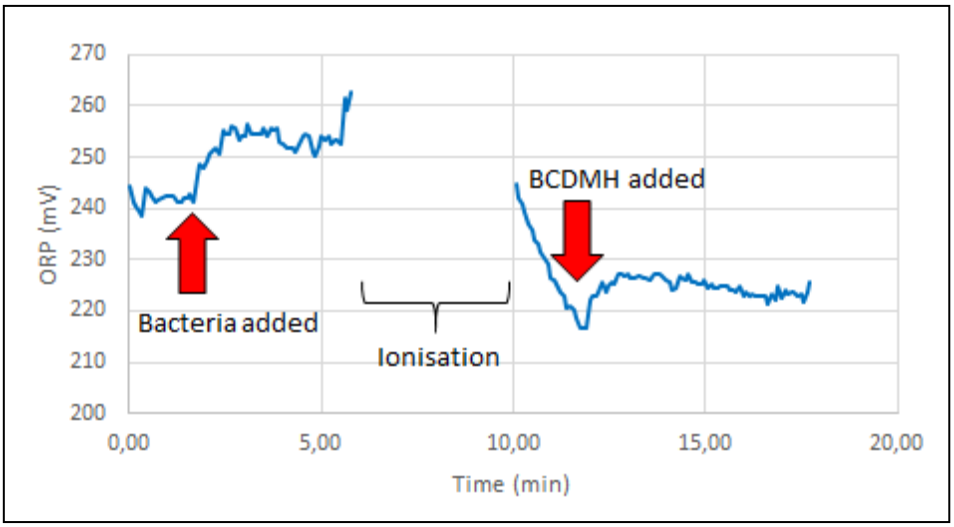

Figure 54: ORP monitor of a typical ionisation-oxidation experiment

When investigating ORP values, there were few significant relationships between ORP and successful disinfection. Table 6 summarises the ORP outputs and the ORP characteristics analysed with logistic regression to investigate any relationship with disinfection success.

Table 6: ORP output models investigated for relationships with successful treatment

ORP characteristic compared with disinfection success	Likelihood score	Model p-value	Cox-Snell R^2
Start ORP	0.814351	0.366837	0.009133
ORP before ionisation	0.357998	0.549620	0.003993
ORP after ionisation	1.036716	0.308587	0.011560
ORP after BCDMH	0.027453	0.868402	0.000305
ORP increase/ decrease	1.937799	0.163908	0.021034
Change in ORP	2.078559	0.149381	0.022801
Change in ORP (+ OR -)	0.952381	0.329114	0.010802
Change in ORP for treatment	0.750412	0.386346	0.008256
Change in ORP for treatment (+ OR -)	1.937799	0.163908	0.021034
Change in ORP for oxidation	5.613166	0.017826	0.063502
Change in ORP for oxidation (+ OR -)	5.022321	0.025023	0.054629

Excluding the change in ORP for the oxidation treatment, all the characteristics analysed were insignificant with p-values >0.05 and likelihood scores <3. The correlation for these models were poor, with Cox-Snell R^2 values being <0.03. The final ORP had no relationship with disinfection success. Final ORP values ranging from 166 mV to 475 mV corresponded to unsuccessful treatment, while final ORP values ranging from 207 mV to 472 mV corresponded to successful disinfection. The change in ORP (ΔORP) for the oxidation treatment was the only output with a significant relationship with

disinfection success. The change in ORP for the oxidation treatment can be described as continuous or as binary, i.e. negative (-) or positive (+). Both approaches to describing the change in ORP for oxidation treatment has a significant relationship with disinfection success, with p-values of 0.018 and 0.025 for the respective models. The continuous model has a smaller p-value and better correlation, with a Cox-Snell R^2 of 0.0635. The continuous model was therefore investigated further. The small Cox-Snell R^2 and small likelihood score demonstrate that the correlation is weak, although significant.

Equation 40 describes the logit model. Figure 55 shows the data for the change in ORP during oxidation treatment and the corresponding treatment success for the different experiments. The probability model for treatment success given a change in ORP is also on Figure 55. The logit model has a significant β_0 that is equal to -0.743139 and has a p-value of 0.001537, and a significant β_1 that is equal to 0.017891 and has a p-value of 0.023582.

$$P = \frac{e^{-9.445+7.158x}}{1+e^{-9.445+7.158x}} \quad \text{(Eq. 40)}$$

Where:

- π = the probability for successful disinfection,
- x = ΔORP (mV).

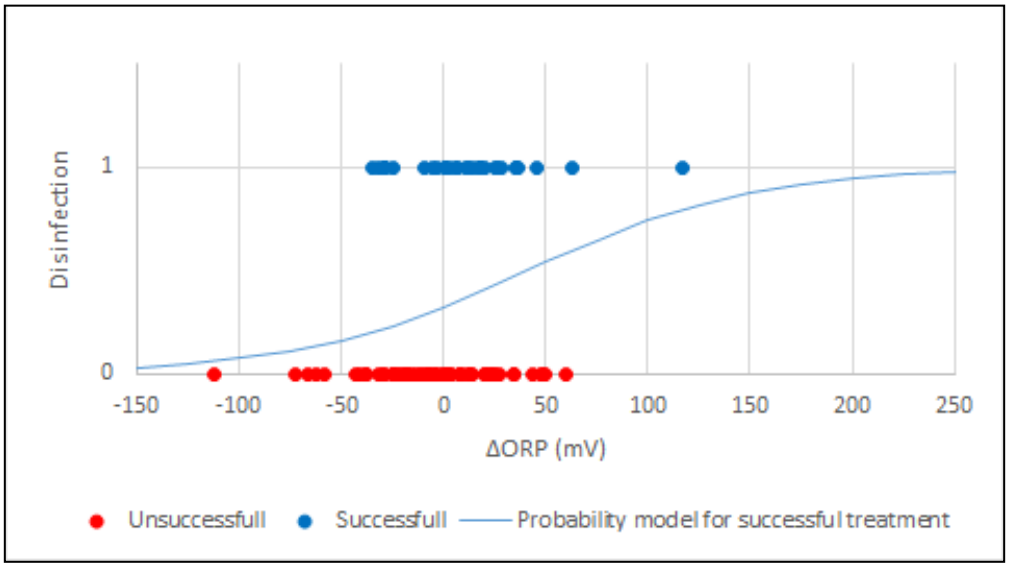

Figure 55: ΔORP for oxidation vs disinfection with probability model

The model has an odds ratio of 1.018, which means the odds for successful disinfection increase with 1.805% for every 1 mV increase in ΔORP. The 90% probability for successful treatment is at a ΔORP of 164.35 mV. None of the experiments caused a ΔORP larger than 117 mV, and therefore further experiments should be done that cause a larger ΔORP for oxidation treatment. The addition of larger

oxidising agent concentrations should cause a further increase in ORP, although the treatment regime also then changes. The logit model has a weak correlation, Cox-Snell R^2 of 0.064, which means that it must be further investigated to ensure its significance. Taking the average ORP value before oxidation treatment into consideration, the aim for final ORP values in further research should be about 480 mV. This should result in final ORP values that could be useful in supporting the results or discarding them.

From the data, a decreasing final ORP has a 49.15% probability of corresponding to unsuccessful disinfection and an increasing final ORP has a 19.80% probability of corresponding to successful disinfection. These relationships are not significant. To investigate ORP as a monitor for disinfection efficiently, experimentation with final ORP values above 450 mV will have to be conducted. The literature that supports ORP as a qualitative indicator for disinfection investigated ORP values above 450 mV. For the experimental treatment, which had final ORP values between 160 mV and 475 mV, no correlation could be found between disinfection and the final ORP. A weakly correlated relationship was found between the ΔORP for oxidation treatment and disinfection success. The ΔORP, could have inconsistencies due to the ORP not given enough time to stabilise between treatment steps.

The change in ORP for oxidation treatment, or even for the complete treatment process, has a higher potential for being a treatment control than the final ORP. Future ORP investigations should put more emphasis on accurate monitoring of initial ORP as well as sufficient stabilisation of ORP between different experimentation steps. It seems if final ORP, as well as ΔORP for different treatment steps, as a tool to monitor disinfection, will correlate better with oxidation-only treatment than with combined ionisation-oxidation treatment.

For the ionisation treatment combined with BCDMH, the control and assessment of disinfection with ORP has several challenges. Firstly, water can have a high ORP even in the absence of sufficient oxidising disinfectants due to an absence of reducing agents. Secondly, the number of different oxidising agents can be present in the water that increases the ORP, but not all of them are necessarily biocidal. Thirdly, the ionisation causes disinfection but decreases the ORP before the addition of BCDMH increases the ORP. Too little experimental data was available to determine whether a smaller amount of BCDMH is needed after ionisation to increase the ORP compared to no ionisation. Finally, the experimental results from the investigation of the combined treatment did not find any correlation between treatment success and final ORP values. The WHO recommended not using a global ORP target, but a treatment specific ORP target (World Health Organization 2008).

4.3.2 Other water characteristics

4.3.2.1 pH

The pH of water cannot serve as an indicator of water pathogens, but is often used in combination with free chlorine measurements to determine the chlorine species in the water. BCDMH treatment involves the release of bromine and chlorine, chlorine's biocidal properties are pH dependent. The pH was therefore monitored to get an understanding of the effect of ionisation and BCDMH treatment on pH, as well as to see whether it affects the correlation between ORP and disinfection success.

Figure 56 shows the typical pH pattern monitored for an ionisation-oxidation disinfection treatment. When the pH probe is initially inserted in the water, it takes a few minutes to give a constant reading, but it stabilises faster than the ORP probe. The sterilised tap water had an initial pH between 6 and 8. The addition of TSB with bacteria usually caused a decrease in pH, while the ionisation caused a slight increase in pH. The additions of the oxidising agent, BCDMH, usually caused an immediate decrease in pH, after which the pH would remain constant, decrease further, or increase slightly once again.

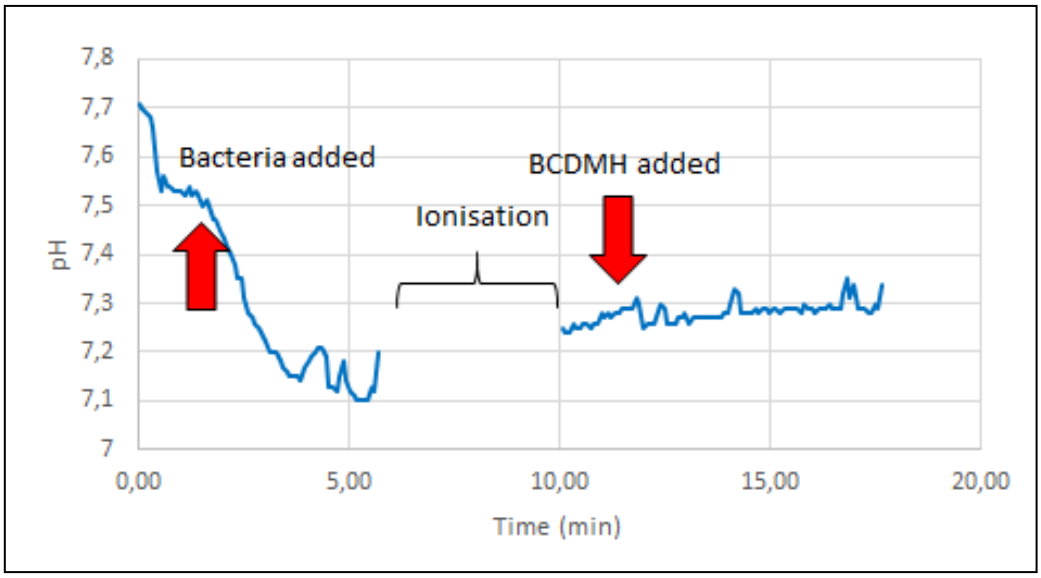

Figure 56: pH monitor of a typical ionisation-oxidation experiment

When investigating final pH values, there was no relationship between pH and successful disinfection. The theoretical relationship could be rejected with a p-value of 0.192 which is >0.05. Successful disinfection was observed at pH ranging from 7.19 to 8.32 and unsuccessful disinfection was observed at pH ranging from 7.08 to 8.62. The pH decreased with the addition of BCDMH, therefore, few successful disinfections were observed at very high pH because it corresponded with low oxidation treatment. There was insufficient free chlorine residual data to investigate free chlorine, pH, and disinfection success. The poor-quality bromine residual data, due to variance in measurements, also

made it impossible to investigate the bromine, pH, and disinfection success. Further investigations should measure free chlorine and bromine residuals accurately for experiments. Residual data should be useful to analyse with pH data to investigate the corresponding ORP, treatment regime and disinfection success.

4.3.2.2 Electric conductivity (EC)

Electric conductivity (EC) is influenced by the salts and other solids in the water. EC is often used to calculate the total dissolved solids (TDS). EC cannot serve as an assessor or control for disinfection on its own, but it can function to help understand the reactions taking place. EC was not expected to be influenced by disinfection treatment, but was monitored to identify any unexpected reactions that might take place in the water.

Figure 57 show the conductivity monitored for a typical ionisation-oxidation disinfection treatment.

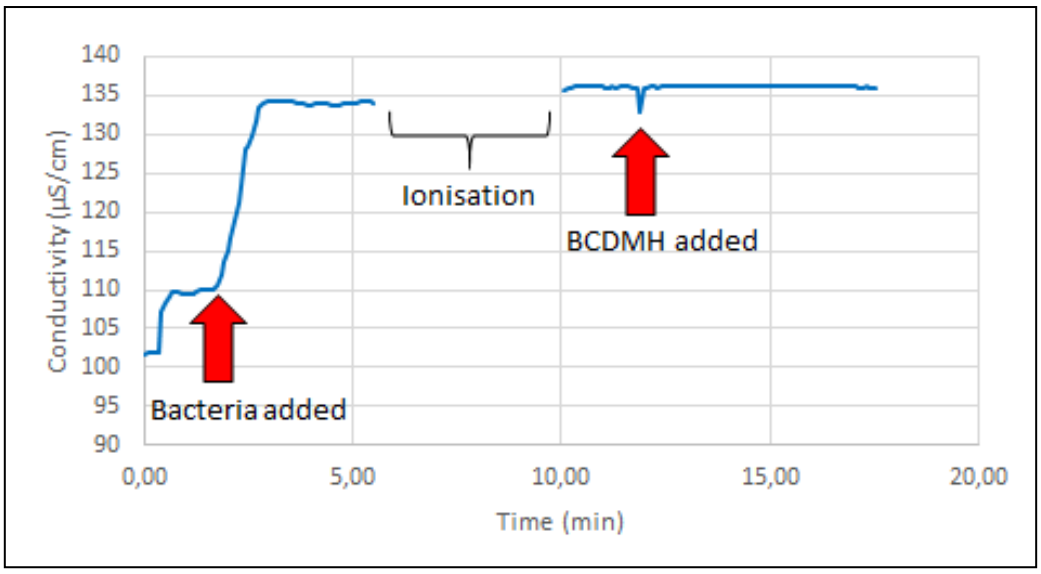

Figure 57: Conductivity monitor of typical experiment

The conductivity reading stabilises quickly, and the sterilised tap water had conductivities that ranged between 80 and 120 µS/cm. The addition of TSB with the bacteria culture caused the EC to increase by between 20 and 50 µS/cm to anything between 105 to 166 µS/cm. None of the disinfection components caused a significant change to the conductivity, not the ionisation or BCDMH addition. EC will therefore not be a control that can be used to monitor the treatment dose for ionisation treatment, oxidation treatment, or the combined treatment.

4.3.2.3 Bromine residual

The Bromine clicker used to measure the bromine residual had such a large variance that the bromine measurements were considered to be very inaccurate. The relationships between the measured

bromine residual, disinfection success, ORP and BCDMH concentration were investigated, but no significant relationship was found. The p-value for the relationship between bromine residual and disinfection success was 0.130 and therefore not significant. Figure 58 shows the measured bromine residual for successful and unsuccessful disinfection, the residuals ranging from 0.2 to 0.8 ppm yielding both successful and unsuccessful disinfection. The measured bromine residuals are far below water quality standards and therefore the treatment is acceptable for potable use as tested under these conditions.

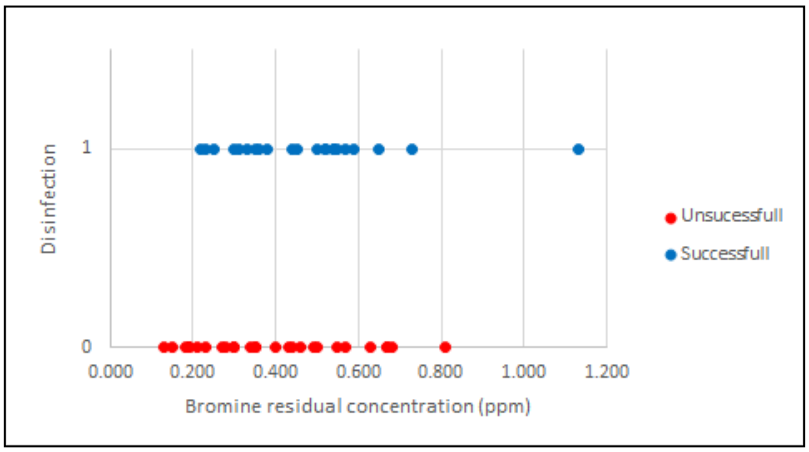

Figure 58: Bromine residual compared to treatment success

The residual bromine measured was expected to be a quantitative indicator of residual disinfectants present and therefore water quality, but it had no significant relationship with disinfection success. Bromine should be valuable if measured accurately instantaneously after disinfection. Relationships between bromine residual and the BCDMH concentration treated, disinfection success, and ORP would be expected. A combined monitor of free chlorine concentration and pH with the bromine residual could link the total halogens available and disinfection success. The single experiment with a high bromine residual showed successful disinfection, a larger variety of experiments with more high and low bromine residuals should give a clearer indication of the bromine monitoring potential.

4.3.3 Summary of assessing disinfection treatment

The investigation into alternative ways of controlling and assessing disinfection did not produce promising results. The oxidation reduction potential (ORP) data did not correlate as strongly with the disinfection success as was expected. The ionisation caused ORP to decrease and the oxidation caused ORP to increase. The change in ORP for the oxidation part of the treatment had a weak, though significant, relationship with disinfection. The final ORP values varied between 160 and 475 mV, but there was no relationship to disinfection success. The final ORP values were, however, below literature values that have been used and are being used to control oxidation treatment. Better monitoring of

ORP data throughout the treatment process and treatment combinations that will yield higher ORP values need to be investigated.

The monitoring of the pH, EC, and bromine residual did not yield any significant results. The pH was monitored to serve as an additional control to help link ORP and bromine residual with disinfection success. The pH increased with ionisation and decreased with BCDMH treatment. The bromine residual data was not significant enough to make any valuable analyses. The pH could therefore also not be combined with the bromine residual and compared to the ORP and disinfection success. The EC was not expected to be influenced by disinfection, and remained basically constant throughout the ionisation and oxidation treatment.

Further research on assessing ionisation-oxidation disinfection should focus on a wider regime of BCDMH concentrations and ionisation intensity. The experiments in this research focused on the transition from unsuccessful to successful disinfection. However, when controls for drinking water are investigated, the subject of interest is only the transition regime, but treatment with a high assurance of being successful. Such treatment could theoretically be assessed and controlled by ORP as the ORP should then be above 600 mV. Accurate free chlorine residual measurements and bromine residual measurements will be helpful to understand the ORP values and its applicability as disinfection control.

5.Feasibility of ionisation-oxidation disinfection

Chapter five combines the literature and experimental results, and discusses the feasibility of ionisation-oxidation water disinfection. Section *5.1 Introduction* explains the purpose of the feasibility study and how it was approached. The different factors that were investigated as part of the feasibility study are then discussed in the sections that follow. *5.2 Disinfection efficiency of combined technology* looks at the disinfecting potential of the ionisation-oxidation process compared to only oxidation. The practicality to implement the technology is discussed in *5.3 Ease of implementation*. Section *5.4 Financial optimisation* looks at the financial implications of the combined technology. The environmental implications of the technology are discussed in *5.5 Environmental footprint*. The feasibility of the combined technology is summarised in *5.7 Summary of feasibility study*.

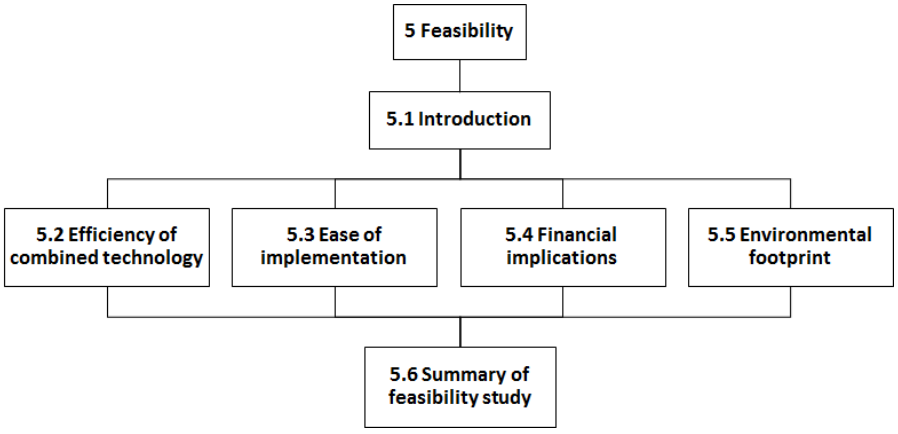

Figure 59: Feasibility structure

5.1 Introduction

Ionisation-oxidation technology has been researched from a scientific perspective and from an application approach. The majority of the scientific surrounding the subject took place in the late 1980s and early 1990s. The research had a consensus that the combined technology was more efficient than the individual disinfectants against a variety of pathogens (Landeen, Yahya et al. 1989, Yahya, Landeen et al. 1990, Pedahzur, Lev et al. 1995, Cassells, Yahya et al. 1995, Fewtrell 2014). Today, ionisation technology is often implemented industrially in combination with oxidising agents, usually chlorine. Advertising commonly claims that ionisation technology decreases the chlorine demand of treated water, such as swimming pools (Carefree Clearwater 2015, Fewtrell 2014). On the South African market, Aquaking makes use of ionisation-oxidation disinfecting technology. Aquaking technology ionises copper, silver, and zinc and combines it with the oxidising agent BCDMH (Aquaking SA 2016). There are, however, scarce literature, data, or reports available on the feasibility of combined ionisation-oxidation technology.

The purpose of the feasibility investigation was to determine whether the metal ion treatment with BCDMH was a feasible alternative disinfectant to chlorine treatment. This is in line with the broader aim of the research and serves to meet the second objective. The overarching aim of the research was to expand the understanding of alternative disinfecting technologies to chlorination, and this was done by focusing on improving the understanding of ionisation-oxidation technology. The second objective of the research was to investigate the feasibility of using metallic ions with BCDMH as disinfectant. The treatment procedure was chosen to represent the Aquaking technology, making the results relevant to the South African market. At the start of the research, the Aquaking technology was known as an effective disinfectant, but there was little scientific research that explained its application (Aquaking SA 2016).

A feasibility study can vary in length and depth. Due to time constraints and the limitations of the scope, the feasibility study serves more as an overview of the applicability of the technology and some advantages and disadvantages. Several factors were investigated to get a broad perspective of the value of the technology. The factors can be categorised broadly into disinfection efficiency, ease of implementation, financial implications, and environmental footprint. Since Aquaking makes use of ORP to monitor and control their system, ORP is discussed as a potential assessment and control to the combined technology (Aquaking SA 2016).

5.2 Disinfection efficiency of combined technology

The aim of a disinfectant is to remove pathogens, i.e. to disinfect. When alternative disinfectants are investigated then one of the most important questions is whether the alternative disinfectant has similar or better disinfecting capabilities than the status quo disinfection. Statements can only be made about the disinfecting efficiency of the combined treatment when compared to a relevant standard. BCDMH treatment on its own was tested and investigated for the same treatment conditions as the combined treatment, and was therefore chosen as comparative standard. This made it possible to compare experimental results and disinfecting models for the combined treatment with BCDMH treatment. This section first compares the experimental results and then discusses the literature to describe the disinfection efficiency of the combined treatment.

The logistic regression model developed for the combined ionisation and BCDMH treatment can be used to understand/determine some disinfecting characteristics regarding the combined process. The model that investigated the combined treatment found the interaction coefficient (β_{12}) to be significant with a p-value of 0.036 and equal to 1.816. The logit model projects that the probability for disinfection success increases with increases in either BCDMH or ionisation treatment. The interaction

coefficient of the model is an indicator that the combined treatment is more effective than the separate processes added together. To understand the gain in efficiency it is worthwhile to compare the model with the BCDMH treatment model.

In chapter four, *Results and discussion*, a logistic disinfection model was developed for the independent BCDMH treatment. The BCDMH logit model and combined treatment model can be compared to understand the efficiency gained by the combined treatment. Figure 60 illustrates the efficiency gained of the combined treatment compared to only BCDMH treatment.

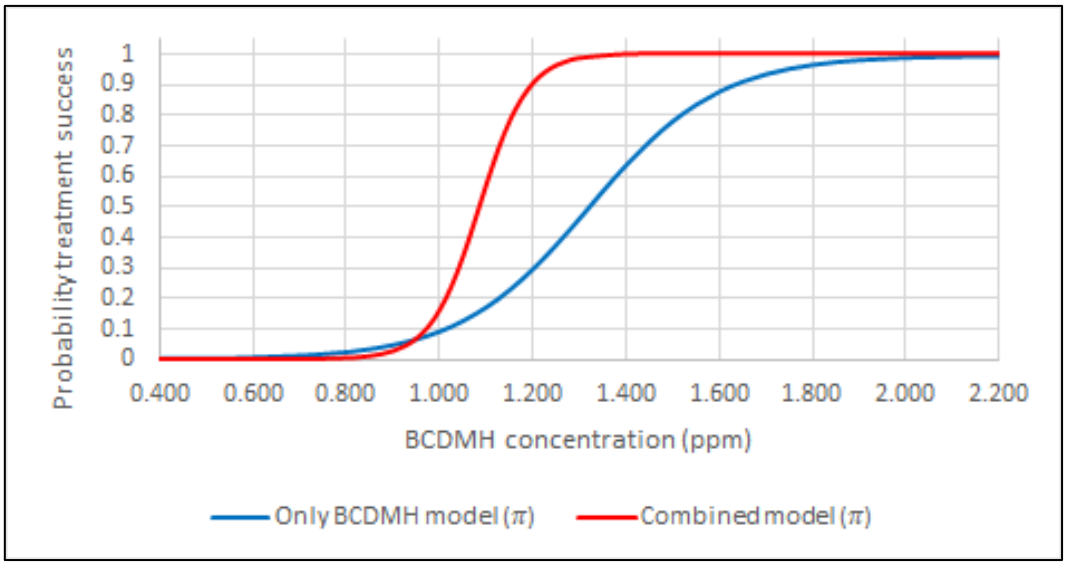

Figure 60: Probability curve for different BCDMH concentrations comparing only BCDMH treatment with BCDMH treatment and 7 coulomb electrons ionised per litre

The blue line represents only BCDMH treatment and the red line represents BCDMH treatment with 7 coulomb electrons ionised per litre. The BCDMH model requires 1.612 ppm BCDMH to have a 90% probability for successful treatment. The combined treatment model requires only 1.198 ppm BCDMH with the 7 coulomb electrons ionised per litre. That is 25.67% less than what the only BCDMH model requires. The shape of the curves is also important to take note of. The combined model has a steeper slope and therefore reaches a high probability quicker than the BCDMH model.

The benefit of the combined technology can easily be described by the decrease in BCDMH used. However, the decrease in BCDMH required would not have been an indicator in efficiency gain if metal ions had a disinfecting capacity for short contact time. Metal ions, however, showed no visible disinfection for any contact time below 30 minutes, no matter how high the ionisation intensity was. Any decrease in BCDMH requirements can therefore be translated into disinfection efficiency gained.

When considering the logistic model for combined treatment, it is valuable to realise that the larger the target probability for successful disinfection, the more BCDMH concentrations can be decreased by the addition of metal ions through ionisation. This can be seen on Figure 61.

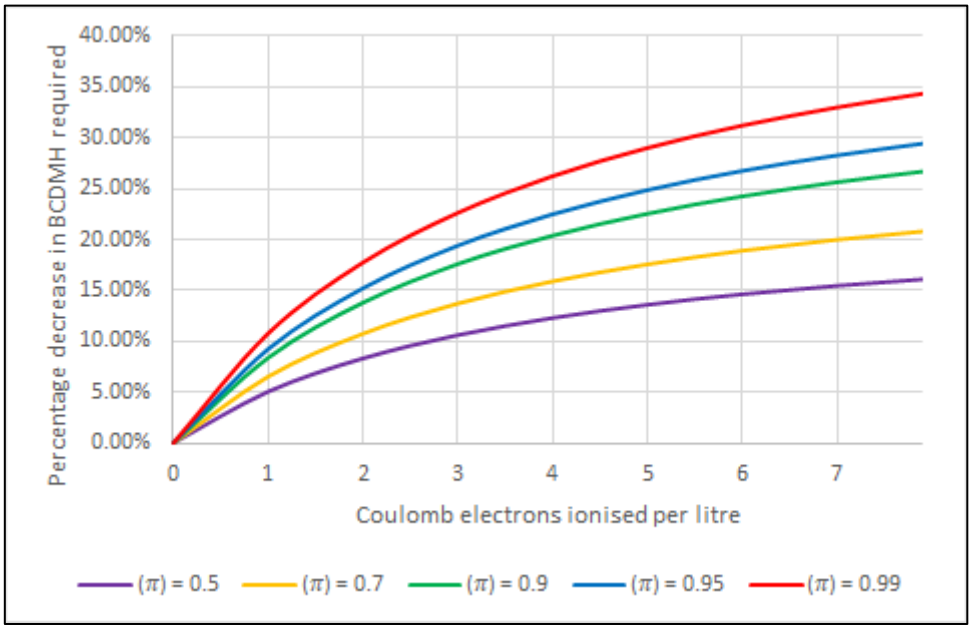

Figure 61: The percentage decrease in BCDMH required vs ionisation intensities for different probabilities successful disinfection

Figure 61 gives the percentage decrease in BCDMH required due to ionisation treatment to reach a certain target probability for successful disinfection. The curves are natural logarithm curves, which increase initially at a high rate and then slowly even out. The purple line is a target probability of 50% for successful disinfection. 7 coulomb electrons ionised per litre can decrease BCDMH concentrations by 15.93% for a target probability for successful disinfection of 50%. For a 99% probability for successful disinfection, BCDMH can be decrease by 32.97%. The decrease in BCDMH required is the result of the gain in efficiency.

According to literature, the combined treatment has several advantages over the individual treatment. Broadly, it was assumed that the combined treatment can be implemented in a way that supports the strengths of both the treatment technologies. This would entail that both disinfectants remain in the water, the usual pathogens are still vulnerable to the treatment and new disinfecting by-products don't form. This needs to be investigated experimentally though. The combination of different metals and an oxidising agent is beneficial in that it consists of a wide variety disinfecting mechanisms, working together, and acting on different structural parts of pathogens (Kim, Anderson et al. 2002, Liu, Stout et al. 1998). The combined treatment consists of bromine, chlorine, silver,

copper, and zinc that can function as biocides. These biocides are different, but seems not to affect each other negatively. Some of the benefits for the combined treatment are discussed below.

Firstly, the combined disinfectant is effective in eliminating a larger variety of pathogens. Due to the mode of disinfection, some pathogens are susceptible to easily be disinfected by certain disinfectants. Other pathogens, such as *Cryptosporidium,* are just not disinfected by common biocides (LeChevallier, Au 2004, McGuire 2006). Chlorine, is generally, effective against most common water pathogens, but has showed limited disinfecting capabilities against certain pathogens, including *Cryptosporidium*, *G. lamblia*, and *legionella* (Lin, Stout et al. 1998, Kelsey 2014, Lin, Vidic et al. 2002, Kim, Anderson et al. 2002). Copper-silver ionisation, on the other hand, has been extremely successful in removing *legionella* from water systems (Lin, Vidic et al. 2002, Kim, Anderson et al. 2002, Liu, Stout et al. 1998). The combined treatment should therefore be more effective against a wider range of pathogens. The same can be seen with biofilm. Chlorine is not as effective in penetrating biofilm, but bromine and copper-silver ionisation have both showed strengths in removing biofilm and disinfecting pathogens within biofilm (Liu, Stout et al. 1998, Liu, Stout et al. 1994, Lin, Vidic et al. 2002, Kim, Anderson et al. 2002).

Secondly, the development of resistant pathogens is less likely as there is always another mechanism that could disinfect. The halogens act as biocides by oxidising pathogens or structural parts of pathogens that lead to pathogen inactivation (Kim, Anderson et al. 2002, Westerlaken 2006). Although bromine and chlorine are both halogens, the oxidising reactions differ so much that bromine is effective against pathogens resistant to chlorine treatment (Walker, Rogers et al. 1994). The metal ions form electrostatic bonds with the negatively charged sites on bacterial cells which lead to pathogen malfunctioning (Liu, Stout et al. 1998). Interestingly, copper, silver, and zinc require different forms of resistant mechanisms to develop in pathogens for resistance (Chudobova, Dostalova et al. 2015). The mutations a pathogen needs to undergo to become resistant to the combined treatment is therefore highly unlikely.

The combined treatment is also less dependent on water characteristics. The disinfecting ability of chlorine is very dependent on the pH of water and that of metal ions have been found to be slightly affected by high pH, but bromine is an effective disinfectant at high pH as well (Walker, Rogers et al. 1994, Lin, Vidic et al. 2002, Leopold, Freese 2009). Bromine and metal ions are both more effective disinfectants in water with ammonia than chlorine (McCoy, Wireman 1989). Changes in temperature should not affect combined disinfection drastically, as metal ions are said to be more effective at higher temperatures while chlorine and bromine is less effective (Lin, Stout et al. 2011, Elsmore 1994, Lin, Vidic et al. 2002, Cachafeiro, Naveira et al. 2007). Metal ions have a residual that has a secondary

disinfecting efficiency that outlasts chlorine and bromine residuals (Lin, Stout et al. 1998, Kelsey 2014, LeChevallier, Au 2004, Liu, Stout et al. 1998, Liu, Stout et al. 1994).

5.3 Ease of implementation

The feasibility investigation should include the practicality of implementing the technology. This section discusses how the combined technology can be implemented and what the consequences are. The points that are discussed include the infrastructure requirements, the operator skill required, the availability of consumables and hazards of implementing the technology. From a practical perspective, it is important to acknowledge that the technology is currently being implemented by Aquaking in South Africa.

The infrastructure needed to do a combined ionisation-oxidation treatment is not too extensive. The Aquaking treatment technology removes a small percentage of water from a reservoir and treats it with a high dosage before releasing it back into the reservoir. The treatment itself requires a footprint of about 1.5 m^2. The cost of the infrastructure to treat would be comparable to requirements for chlorination. The technology can also be implemented in-line, although the fluctuations in water quality can then not be considered. The consumables, namely metal electrodes and BCDMH, are available on the South African market. BCDMH is, however, imported from China with limited local suppliers.

The skill required to operate the combined treatment will be dependent on the controls put in place to assess disinfection. The Aquaking treatment is monitored and controlled by the ORP, which results in an automated treatment process. In such cases operators only need to check chemicals and electrodes periodically to ensure treatment is done correctly. Operators also need to check the general water quality tests that ensure water is within the acceptable standards, these will include periodic *coliform* tests (Lin, Stout et al. 2011). The combination treatment can also be controlled by measuring free chlorine residual and bromine residual. The operator skills required would then be comparable to the skills required to run a chlorinator.

The combined treatment has some other operational advantages. Metal piping, reservoirs, etc. are prone to corrosion when excessive chlorine or bromine is present in water (Kelsey 2014, Lin, Vidic et al. 1998, Lin, Stout et al. 1998). The decrease in chlorine and bromine due to the metal ions can lead to a decrease in corrosion which has financial and operational benefits. Oxidising agents are hazardous and dangerous to handle, store and treat with, but BCDMH is stable in its tablet and powder form. Workers handling BCDMH or metal electrodes are not exposed to extreme risk. The highest safety risk

involves electricity used in close proximity to water, but safety precautions can be put in place to decrease the risk.

5.4 Financial implication

Financial benefits and optimisation play a critical role when investigating alternative technology. The financial effect of alternative technology often becomes the final factor that leads to decisions being made. The financial implications and optimisation of the combined ionisation treatment with BCDMH were therefore investigated. Extensive economical investigations can be done that investigate the infrastructure required, maintenance and operational expenses. Due to the time available and scope of the research, the financial investigation was simplified to focus on the operational cost. The infrastructure and maintenance expenses would be unique for every treatment plant.

The operational cost was defined as the cost of consumables used and that needed to be replaced for the treatment to take place. For the ionisation the metal electrodes are used and for the oxidation BCDMH is used. Treatment costs are specific to the volume of water treated. Costs were calculated in cents per kL treated using current market prices for BCDMH and metal.

Table 7 contains the market prices for BCDMH from local suppliers (Aquaking SA 2017), and copper, silver, and zinc as given by The World Bank for June 2017 (The World Bank Group 2017). Silver is the most expensive of all the consumables that form part of the treatment, and although little of it is used in ionisation, it plays a critical factor in the operational cost. For the treatment of 1 litre water, 1 mg BCDMH is required to increase the BCDMH treatment concentration by 1 ppm. The earlier developed Equation 39 can be used to calculate the loss of metal mass per coulomb electron ionised. Treatment costs increase by 23.14c for an increase in 1 ppm BCDMH treatment per kL water. For every coulomb electron ionised per litre, treatment costs will increase by 9.37c per kL water treated.

Table 7: Cost of metals and BCDMH treatment

Disinfectant	Price per kg (Rand)	Price per gram (Cents)	mg used per BCDMH ppm	mg used per coulomb electron ionised per kL treated	Cost per ppm BCDMH treatment per kL (cents)	Cost per coulomb electron treatment per kL treated (cents)
BCDMH	R231.42	23.14	1		23.142	
Copper	R73.84	7.38		286.9480132		2.119
Silver	R7 027.18	702.72		10.12757694		7.117
Zinc	R33.22	3.32		40.51030774		0.135

The cost model is dependent on the BCDMH and metal electrodes used. The logistic regression model were created to predict the disinfection combination required to achieve a probability for successful

disinfection. Figure 62 recaps the combination relationship between BCDMH concentrations and ionisation to achieve different probabilities for successful disinfection.

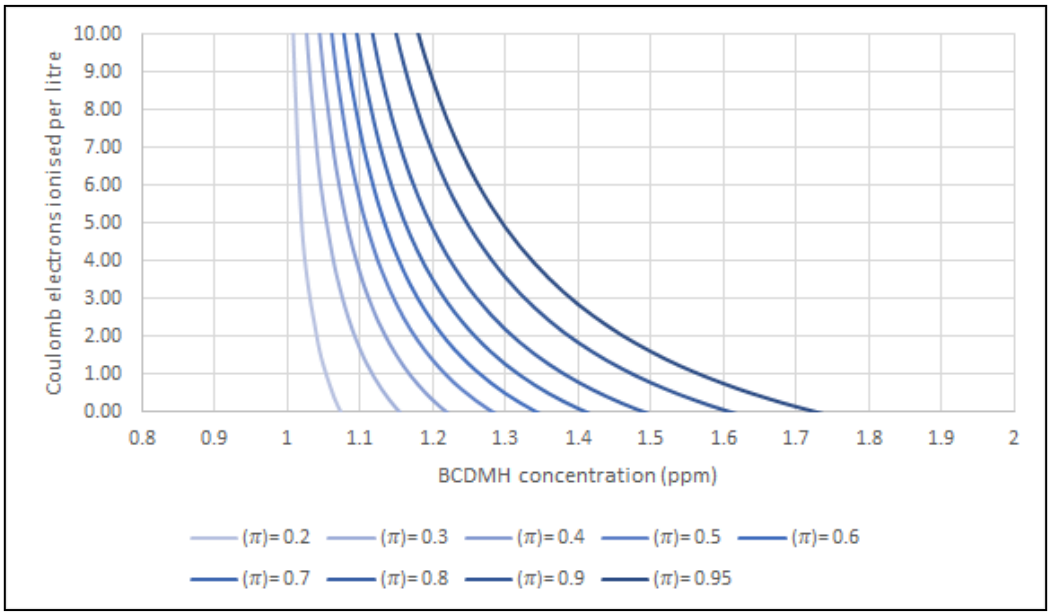

Figure 62: Probability for successful disinfection

This relationship is used to calculate the operational cost curve. The cost curve can be created for different probabilities for successful disinfection. Figure 63 represents the cost for treatment combinations for a 90% probability for successful treatment.

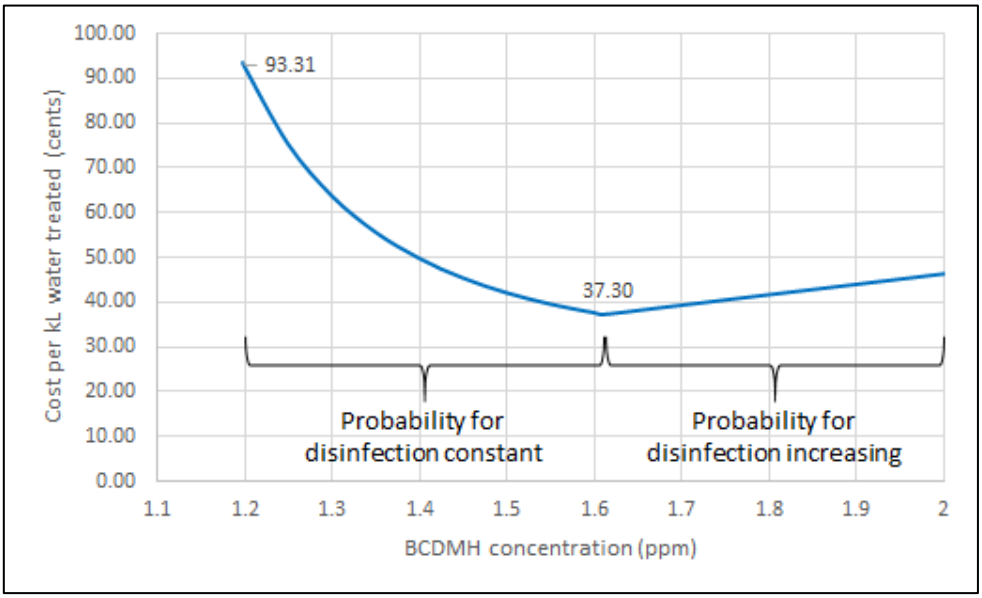

Figure 63: Cost for 90% probability for successful treatment

For the lowest amount of BCDMH concentration the most ionisation is required and the treatment is then the most expensive combination plausible. The ionisation component is far more expensive than

the oxidation part. The lowest cost treatment point for 1 kL water is 37.30c with no ionisation and only BCDMH treatment. A minimum amount of BCDMH and maximum amount of ionisation will cost of 93.31c per kL treated. Therefore, a decrease in 25.67% BCDMH that can be attained, will come at a cost 2.5 times more than using only BCDMH as disinfectant. Lower probability investigations lead to lower costs, but also smaller amounts of ionisation which causes a lower decrease in BCDMH required. The cost of the metals undermines the advantages of the combined treatment to a degree.

The economic feasibility has really been simplified to identify the possible economic implication of the combined technology. A more complete investigation should include comprehensive studies of all the possible expenses of the individual treatment processes and the combined process. The capital input and infrastructure development required will have to be investigated with the lifespan of the technology. There are also other operational costs that can be investigated, such as operator salaries, operator skill development and control costs. A more complete investigation will have to be done as a case study due to all the case specific variables that influence the cost of the treatment. The application of the combined system will be implemented at higher concentrations to ensure water quality. The excessive treatment could influence the financial feasibility of the combined treatment.

5.5 Environmental footprint

The need to treat water is partly due to the poor management of water and other resources in the present and past. How ironic that the treating procedures could cause other negative effects to the environment and to humans. One of the leading factors that promote the development of alternative disinfectants to chlorination is the negative health effects chlorine treatment can have on humans and the environment. First the theoretical difference in the environmental impact is discussed between combined treatment and chlorine disinfection. Then the potential decrease in oxidising chemicals are discussed for the combined treatment.

Chlorine treatment is known to have several potential negative effects on humans and the environment. Hence forth the aim of water providers to decrease chemicals used for disinfection while ensuring a constant water quality. The formation of trihalomethanes (THMs) is one of the most serious health risk to humans. THMs form when halogens, especially chlorine and bromine, react with organic material in water, THMs are carcinogenic (Lin, Vidic et al. 1998, Lin, Stout et al. 1998, Kim, Anderson et al. 2002). An excess halogen disinfectant or large concentrations organic matter usually enhance the formation of THMs. Chlorine and bromine overtreatment can cause direct health issues. Excessive intake of bromine, for example, can cause nausea, vomiting, abdominal pain, and under extreme conditions, even paralysis and coma (Kim 2014, WHO 2009). Oxidising chemicals also affect the

palatability of water as treated water often smells and tastes like chemicals. The release of treated water back into natural water sources have the potential to have various adverse effects on aquatic, microbial and even terrestrial life.

The combined treatment of metal ionisation with BCDMH also has the potential to affect humans and the environment. BCDMH contain both bromine and chlorine that can have the same health risks as when used individually for treatment. The BCDMH, however, ensures that both chemicals are present at lower concentrations. The addition of metal ions does not necessarily improve the environmental footprint, but has the potential to decrease the negative effect. The ionisation model investigated treated water with metal concentrations below the SANS-241 limits, but the effect of human exposure to copper, silver and zinc is still largely unknown (Zheng, Dunets et al. 2012). The metal ions residual is known to be present for a long time after treatment, therefore, the effect ionisation could have on the biotic communities in natural water bodies could be immense. However, at this point the consequences of ionisation is still not understood.

The combined disinfection has the environmental benefit of a reduced amount of oxidising agent used. The logistic regression model developed for combined treatment showed that the more ionisation is used for treatment the less oxidising agents need to be used to reach the same probability for disinfection success. Figure 64 show the maximum percentage decrease in BCDMH required for treatment for different probabilities for successful treatment.

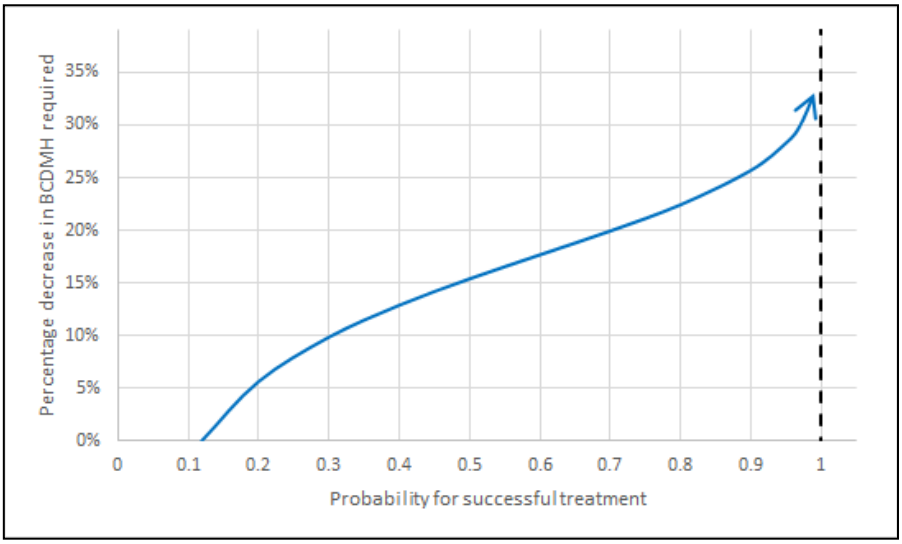

Figure 64: Maximum percentage decrease in BCDMH used for different probabilities for successful disinfection

The maximum decrease in BCDMH is due to the additional maximum allowable ionisation treatment, i.e. 7 coulomb electrons ionised per litre. From the graph the percentage decrease in BCDMH required to reach a target probability increases as the target probability increases. The curve is very linear

between target probabilities of 0.4 and 0.85, thereafter the change in probability causes a rapid increase in in the reduction of BCDMH required.

Ionisation can decrease BCDMH required by 26.83% for 90% probability for successful treatment. For a 95% probability for successful treatment, BCDM required can be decreased by 28.27%, and for a 99% probability for successful treatment, BCDMH required can be decreased by 32.97%. With the specific focus of decreasing oxidising chemicals for human health, the combined technology becomes a valuable alternative. For the experimental conditions the metals precipitate out and should not build up to become a health issue. This should however be researched thoroughly. Metals will not cause the formation of THMs or other high risk oxidising by-products.

5.6 Summary of feasibility study

To summarise, the metal ionisation treatment combined with BCDMH treatment has several advantages. There is strong evidence that the combined technology is more efficient than the individual treatments alone. The combined treatment should be more efficient to disinfect a wider range of pathogens as well. The implementation and operation of the process does not seem more complicated than chlorination and will not be a limitation to the process. Oxidising chemicals can be decreased up to 25.67% for a target probability of 90% for successful disinfection. The decrease in chemicals used has a beneficial environmental impact and decreases the human health risk due to exposure to chemicals. The assessment and control of the combined treatment process have similar challenges to the assessment and control of chlorine treatment.

The combined technology also has some disadvantages. The main drawback is that the combined treatment is more expensive than BCDMH-only treatment. For a target probability of 90% for successful disinfection the 25.67% decrease in BCDMH required would come at a cost 2.5 times more than when only BCDMH is used as treatment. The actual interaction involved between the metal ions and oxidising agents are unknown and may cause problems if implemented in an incorrect way. BCDMH contain both chlorine and bromine, but bromine is thought to be carcinogenic and can cause health problems if over exposure occurs. Poor treatment control will have adverse effects on humans consuming the water.

The combined technology shows a lot of promise as an alternative disinfectant. According to the feasibility investigation, there are several benefits of the combined technology, but this comes at a significant higher cost. The question is how important is it really to decrease the amount of oxidising chemicals being used. The feasibility can also be improved by investigating the interaction effects on all the different factors without discussing it only out of literature. The feasibility investigation focused

on the technology as a disinfectant, but the process could also have other benefits in water treatment outside the scope of disinfection. A final consideration is that Aquaking has found their technology to be very effective in the poultry and fruit packing industries, and to treat cooling tower water supply (Aquaking SA 2016). The applicability of the technology should be continued to be investigated in the lab and at industrial sites to determine the strengths and limitations of the process. A more complete understanding of the strengths and limitations will lead to enhancing the technology and implementing it in applications where it will be effective.

6.Conclusions and recommendations

6.1 Summary of research

The objectives of the research were (i) to identify the contribution, if any, of metallic ions on the disinfection ability of BCDMH. (ii) To investigate the feasibility of using metallic ions with BCDMH as disinfectant. (iii) To evaluate ORP as an indicator of disinfection efficacy for a disinfection process that combine metal ions with BCDMH.

A lab scale batch experimental apparatus was developed to be used to test the effectiveness of BCDMH and metal ions as biological disinfectants for point of use water, both separately and in combination. Disinfectants were tested against water artificially contaminated with bacterium *Pseudomonas sp. strain CT07* at a concentration of between $0.5x10^7$ and $2.0x10^7$ cfu/ml. Disinfection was defined as reducing the bacterial concentrations to below detectable levels using TSA plating. The experimental treatment consisted of an ionisation component and an oxidation component. The ionisation released copper, silver, and zinc ions as disinfectants through electrolysis. A BCDMH stock solution was used as an oxidising agent. Treatment was conducted with a 5-minute contact time to represent point-of-use disinfection.

BCDMH and metal ionisation were first independently investigated as disinfectants to use as references for the combined treatment. BCDDMH and metal ionisation was then investigated as a combined treatment. BCDMH concentrations between 0.75 ppm and 1.60 ppm were investigated with metal ions released due to ionisation ranging from 0 to 7 coulomb electrons ionised per litre. The data was analysed using logistic regression to develop probability models for successful disinfection. Throughout experimentation, ORP was monitored in order to investigate the correlation between ORP, BCDMH concentration and probability of successful disinfection.

Batch experimentation showed that BCDMH required a 5 minute contact time for successful disinfection. Complete disinfection was possible in less than 5 minutes, while no bacterial reduction took place after 30 minutes contact time. The disinfection data on only BCDMH treatment for a 5-minute contact time was analysed with logistic regression to give Equation 41:

$$P = \frac{e^{-9.445+7.158x}}{1+e^{-9.445+7.158x}} \qquad \text{(Eq. 41)}$$

The data fitted a logistic regression model well with a Cox-Snell R^2 of 0.601 and a p-value <0.001. The results showed that a BCDMH concentration of 1.63 ppm had a 90% probability for successful treatment. The model developed, further showed that an increase in 0.1 ppm BCDMH treatment

increased the odds for successful disinfection by 104.59%. BCDMH can be used as a point-of-use water disinfectant.

Ionisation of copper, silver and zinc proved successful for disinfection, but only after an extensive contact time. For metal concentrations below the SANS-241 health limits, a contact time of at least 60 minutes was needed to disinfect successfully. Metal ionisation would not be a plausible point-of-use water disinfectant.

The combined treatment results were analysed with logistic regression to formulate Equation 42:

$$\pi = \frac{e^{-8.529+6.654x_1-1.782x_2+1.816x_1x_2}}{1+e^{-8.529+6.654x_1-1.782x_2+1.816x_1x_2}} \quad \text{(Eq. 42)}$$

The model had a strong correlation with a Cox-Snell R^2 of 0.516 and was significant with a p-value <0.001. The interaction coefficient (β_{12}) was significant with a p-value of 0.036. The significant interaction coefficient showed that metal ions could improve the disinfecting ability of BCDMH at an acceptable contact time of 5-minutes. The addition of metallic ions decreases the amount of BCDMH required to attain a certain probability for successful treatment according to the probability model.

The monitoring of the disinfection process yielded no significant results in the relationship between the final ORP values and the disinfection success achieved. The pH and EC measured during experimentation did not provide any understanding into ORP changes relevant to the disinfection process. The monitored ORP and pH followed patterns during the treatment process while the EC remained constant for ionisation and BCDMH treatment. The change in ORP for the BCDMH treatment showed a significant relationship with treatment success with a p-value of 0.018. The relationship showed that a ΔORP of 164.35 mV should correspond to a 90% probability for successful treatment, although the maximum experimental ΔORP was 117 mV. The variability of initial ORP values and final ORP values below 475 mV could have affected the relevance of the monitored ORP data. The monitored ORP seldom went above 450 mV and a threshold ORP could not be determined, both indicators referred to in literature.

The feasibility of the combined technology considered the disinfection efficiency, ease of implementation, financial optimisation, and environmental implication. The combined process seems to be more efficient, can easily be implemented, and has environmental benefits. However, the combined technology is more expensive to operate than BCDMH treatment independently. The addition of metal ions through ionisation can lead to a 25.67% reduction in BCDMH used, but it costs 2.5 times more than treatment without metal ions. Case specific investigations will determine whether the cost of the combined technology is justifiable.

To summarise, the research found a significant disinfection interaction between metal ions and BCDMH. The combined treatment was more efficient than the individual processes added together. The combination of metal ions and BCDMH showed higher disinfection efficiency, but the gain comes at a financial cost. The final ORP values could not be related to disinfection success, although the change in ORP for the BCDMH part of treatment correlated weakly with treatment success.

6.2 Recommendations for future research

The study has determined several areas as directions for future research.

1. The experimental methodology can be improved to have better control over the feed and treatment components. A variety of feeds and pathogens can be investigated to identify the effect of disinfection on high risk pathogens. More exact control of metal concentrations and BCDMH concentrations can help to understand the mode of disinfection and chemical reactions taking place. Research into continuous treatment and treating natural water sources will yield results that can help with feasibility. A full CT investigation for the combined treatment will show a true perspective of the interaction between metal ions and BCDMH.
2. Different metals and metal combinations can be researched with different oxidising agents. The contribution different metals have on disinfection could be of value. It would require an alternative analysis method such as ICP-MS to determine metal concentrations. Other oxidising agents, such as ozone, could show higher efficiency than halogen based oxidising agents and could be tested in conjunction with the ionisation process in a similar manner to the current work. The combined ionisation-oxidation can be investigated on other water contaminants, such as heavy metal, low levels of salinity, and hardness. These might broaden the technology's application range.
3. The ORP results do not seem to warrant further research, although literature seem to promote further research in the ORP direction. Disinfection experiments that result in higher ORP values should validate the use of ORP as indicator of disinfection efficacy. Proper control and measuring of free chlorine and bromine residuals can serve to help understand ORP values in relations to BCDMH treatment and disinfection success. Determining threshold ORP values can broaden the application potential of ORP technology.

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Appendix A – Experimental equipment

Microbiology equipment

In the microbiology laboratory, there is a wide variety of equipment. Some of these are more general laboratory equipment and other things are specific to microbiology work. This section will document the types, purpose, brands, and operating parameters of the experimental equipment employed.

In the microbiology laboratory, there were conditions that simplified the experimentation. The working bench had a Bunsen burner, Figure A1, which was supplied with natural gas that was used to do aseptic work. The white backlight, Figure A2, was used to count bacterial colonies on petri dishes. There were two incubation rooms, one kept at 37°C and the other at 30°C, with drying racks and shakers. The drying racks were used to dry plates and the shakers were used to continuously mix the bacteria cultures growing in liquid media. Figure A3 is a photo of the shaker found in the 30°C incubation room and figure A4 is a photo of the drying rack in the 37°C incubation room.

Figure A1: White-backlight for plate counting

Figure A2: White-backlight for plate counting

Figure A3: Shaker

Figure A4: Drying racks

A Vortex-Genie 2, manufactured by Scientific Industries, were used to vortex, i.e. mix or shake, the Eppendorf tubes. The vortex has a “ON”, “OFF” and “TOUCH” function. The “TOUCH” function was used to vortex for about 5 seconds. Figure A5 is a photo of the vortex. A HICLAVE HV-85 autoclave was used to autoclave, i.e. sterilise, equipment and consumables. Figure A6 is a photo of the autoclave. A Fried Electric magnetic stirrer, figure A7, was used to stir the experimental solution.

Figure A5: Vortex

Figure A6: Autoclave

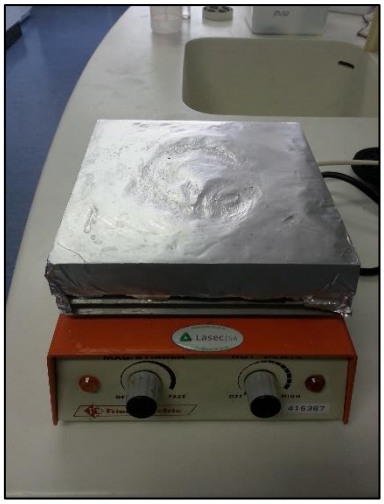


Figure A7: Magnetic stirrer

Pipettes, measuring cylinders and syringes were used to measure, remove, or add specific volumes of liquid. For volumes between 20 µL and 200 µL the Gilson Pipetman P200 was used, for volumes between 200 µL and 1000 µL the Gilson Pipetman P1000N was used. Figure A8 is a photo of the pipettes. The automated Labnet Excel Electronic Pipette, figure A9, was used for plating. Figure A10 is a photo of the Glassco measuring cylinders that were used. The 1000-mL measuring cylinder has a variability of ±5.0 mL, the 500-mL cylinder has a variability of ±2.5 mL, and the 100-mL cylinder has a variability of ±0.5 mL at 20°C.

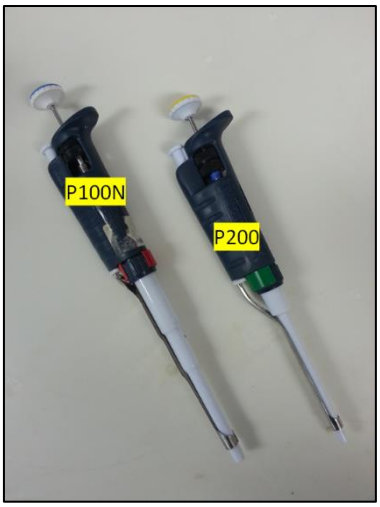


Figure A8: Vortex

Figure A9: Automated pipette

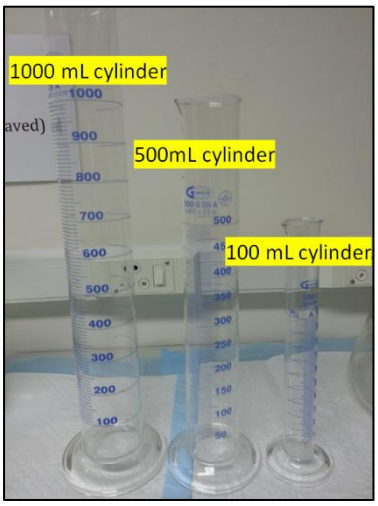


Figure A10: Measuring cylinders

To weigh substances two different scales were used. The Metler AE160 has a 0.1 mg resolution and was used to measure masses below 10 g. Figure A11 is a photo of the Metler AE160. The Radwag PS750/C/1, figure A12, was used to measure masses above 10 g. A Manson NSP-6016 power supply was used to supply direct current for experimentation, figure A13. The NSP-6016 can be used to supply an adjustable output voltage ranging from 0 to 60V or an adjustable output current from 0 to 1.6 A.

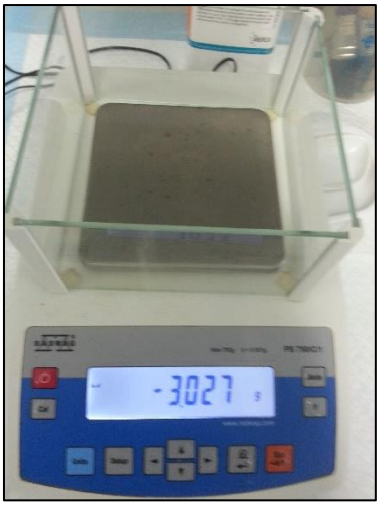


Figure A11: Vortex

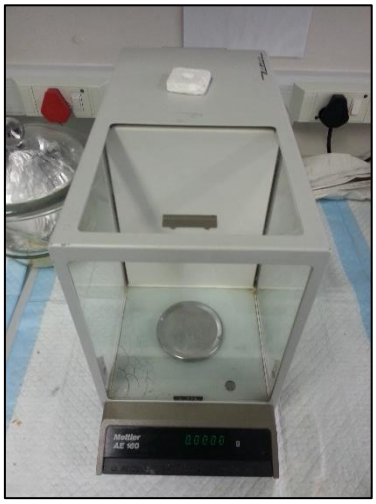


Figure A12: Automated pipette

Figure A13: Measuring cylinders

A variety of other glassware were used. 5L, 3L and 1L Erlenmeyer flasks were used to sterilise liquid in the autoclave and for bacterial growth. 1000 mL, 500 mL and 50 mL glass bottles were used to store liquids and solutions and to sterilise liquids that were to be stored. Figure A14 is a photo of a 250-mL Erlenmeyer flask and a 500-mL glass bottle. Other general microbiology equipment used include a mortar and pestle, Eppendorf tube holders and pipette tip holders.

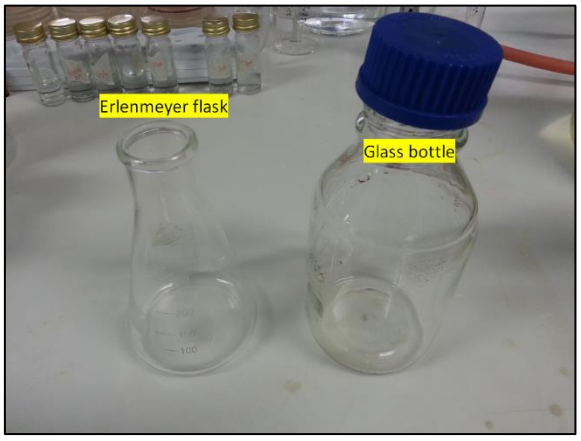


Figure A14: Erlenmeyer flask and glass bottle

Analytical equipment

The analytical equipment is specific equipment that was used for monitoring or reading or quantifying during experimentation. The specifications, accuracy and range of the different equipment used is given.

A Merck Spectroquant® Pharo300 spectrometer was used to measure the absorbance, or optical density (OD). The absorbance had a standard deviation of 0.009 when the OD_{600} was measured for a bacterial culture grown for 17 hours with an average OD_{600} of 0.839. Figure A15 is a photo of the spectrometer. The ISP-MS equipment used is a Thermo Scientific iCAP6200 Spectrometer with Qtegra software. An internal standard method with Yttrium was used as internal standard. A fluke 179 True RMS Multi-meter was used to monitor the current supplied to the ionisation. The Fluke 179 measures current to a resolution of 0.01 mA. The multi-meter can be seen in figure A16.

Figure A15: White-backlight for plate counting

Figure A16: Fluke 179 multi-meter

Several of the monitoring equipment implemented have been manufactured by Hanna Instruments. A HI 716 Bromine Checker was used to measure bromine residual. The HI 716 can measure bromine concentrations between 0.60 and 8.00 ppm with a 0.1 ppm resolution and an accuracy of ± 0.08 ppm or ± 5% of the reading, whichever is more. Repeatability experiments showed the standard deviation was 18% on samples with an average bromine concentration of 440 ppm. A HI 701 Free Chlorine Checker was used to measure free chlorine concentrations. Repeatability experiments showed a standard deviation of 14% on samples with an average free chlorine concentration of 162 ppm. Figure A17 is a photo of the Bromine Clicker and figure A18 is a photo of the free chlorine Clicker with the standards used to check calibration.

Figure A17: HI Bromine Checker with standards

Figure A18: HI Free Chlorine Checker with reagent

The ORP was monitored with a Hanna Instruments edge® pH meter and HI36180 ORP probe. The combination has a range of ±2000 mV, a resolution of 0.1 mV, and an accuracy of ±0.2 mV for readings ±999.9 mV and ±1 mV for readings ±2000 mV. The pH was measured with a Hanna Instruments edge® pH meter and HI11310 pH probe. The combination has a range from -2.00 to 16.00 pH, a resolution of 0.01 pH, and an accuracy of ±0.01 pH. The electric conductivity was measured with a Hanna Instruments edge® EC meter and HI763100 EC probe. Depending on the calibration done, the combination can be used to measure EC from 0.00 µS/cm to 500.00 mS/cm. The resolution and accuracy changes depending on the calibration and EC measured. For electric conductivity between 30.0 and 299.9 µS/cm, the probe and meter measures EC up to 0.1 µS/cm at an accuracy of ±1% of reading. Figure A19 is a photo of the meters and figure A20 is a photo of the probes.

Figure A19: ORP, pH and EC meter

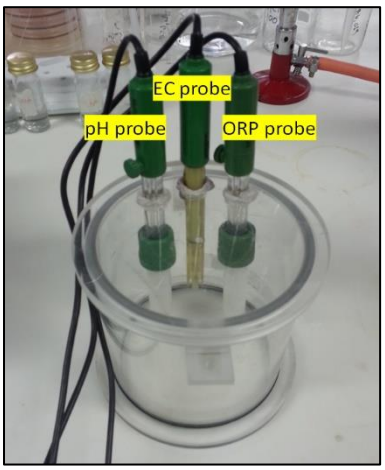


Figure A20: ORP, pH and EC probes

Consumables and chemicals

A distinction has been made between equipment and consumables. The consumables include everything that can only be used for a limited number of times before it must be replaced. The specifications of the consumables are given.

With the P200 pipette, small yellow pipette tips were used, while large blue pipette tips were used with the P100N pipette. Different size Eppendorf tubes are available, but the 1.5 mL tubes were used for dilutions and freezer cultures. 10 mL and 20 mL syringes were used for the measuring of and removal of liquids between 5 and 20 mL. 0.22 µm syringe filters were used to filter samples that had to be analysed by the ICP-MS. 15 mL Falcon tubes were used to keep ICP-MS samples. To measure the absorbance in the spectrometer, disposable plastic cuvettes were used. Standard sterile petri dishes were used for plating. Other general consumables used include tin foil, tissue paper, and parafilm. Figure A21 is a photo of some of the consumables used.

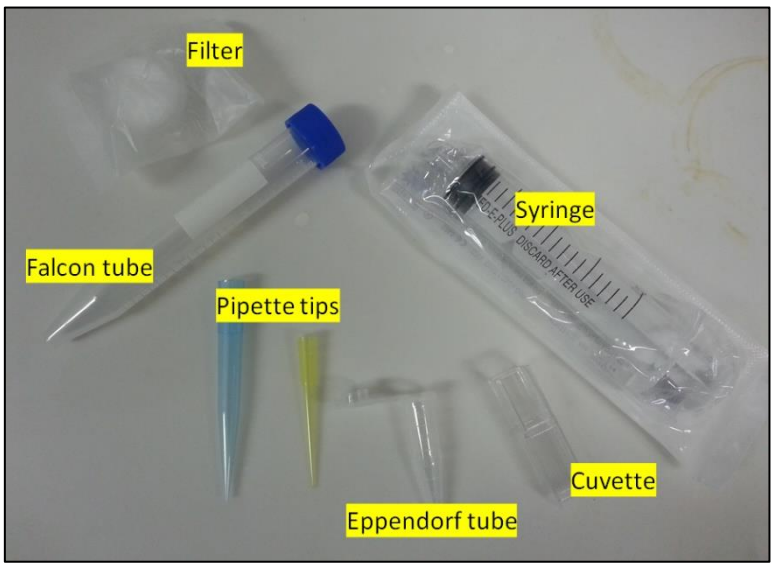


Figure A21: Some of the consumables used

Different chemicals were used for the general microbiology procedures and for the treatment procedure. The suppliers and general application of the different chemicals are described in the following section where relevant.

For the microbiology procedures Nutrient Agar and Tryptone Soy Broth (TSB), both produced by the Biolab, were used. Nutrient Agar was used to make solid media (TSA) and TSB was used to make liquid media. Sodium chloride (NaCl), CAS number 7647-17-5 and produced by Sigwa-Aldrich, was used to make saline solution. Glycerol ($C_3H_8O_3$) was used for to make freezer cultures to be stored.

For sterilisation purposes, either a 30% ethanol (C_2H_6O) mix or bleach were used. The 30% ethanol was made from RO water and 95% ethanol and stored in a spray container. Chlor Guard Sanitizer Bleach, with 3.5% active sodium hypochlorite (NaClO), was used as bleach. Nitric acid (HNO_3) was used to dissolve solutions for ICP-MS analysis. Bromor-chloro-dimethylhydantoin (BCDMH) ($C_5H_6BrClN_2O_2$) was used as oxidising agent. The Free Chlorine Checker used dpd reagents sold as HI 701-25 and the Bromine Checker used dpd reagents sold as HI 701-25.

Accuracy of equipment

Table A1: Raw data for determining Bromine and Free Chlorine standard deviation

Determining Clicker repeatabiltiy for measurements of BCDMH stock solution							
Time:	Age (hours)						
12:00	20:00						
	Bromine concentration (ppm)						
Sample	R1	R2	R3	R4	R5	Average	Std. Dev.
S1	577	559	539	533	506	542.80	26.91
S2	406	400	393	388	383	394.00	9.19
S3	445	443	439	435	424	437.20	8.32
S4	539	488	485	480	483	495.00	24.77
S5	340	331	330	326	329	331.20	5.26
				Average	440.04	440.04	440.04
				Std. Dev.	77.42	83.00	26.91
				% Std. Dev	0.175935	0.188613	0.061156
	Free chlorine concentraion (ppm)						
Sample	R1	R2	R3	R4	R5		Std. Dev.
S1	143	149	155	158	162	153.40	7.50
S2	179	211	207	203	202	200.40	12.48
S3	135	138	140	143	147	140.60	4.62
S4	146	163	171	176	184	168.00	14.47
S5	144	145	147	149	149	146.80	2.28
				Average	161.84	161.84	161.84
				Std. Dev.	23.42	23.84	14.47
				% Std. Dev	0.144686	0.147306	0.089435

Data and tables and graphs

Table A2: Raw data for determining OD_{600} standard deviation

100 ml TSB was innoculated at 9:00 pm with a scoop from a plate on 15 Feb							
5 samples were taken and each measured 5 times at 2:00 pm the next day (i.e. 17 hours later), 16 Feb							
	R1	R2	R3	R4	R5	Average	Std. dev.
S1	0.860	0.838	0.855	0.832	0.835	0.844	0.013
S2	0.859	0.841	0.833	0.829	0.830	0.838	0.012
S3	0.847	0.841	0.843	0.841	0.838	0.842	0.003
S4	0.835	0.845	0.829	0.827	0.835	0.834	0.007
S5	0.845	0.837	0.835	0.841	0.829	0.837	0.006
ref	0.003	0.002	0.008	0.011	0.006	0.006	0.004
						Average:	0.839
						Std. dev.	0.009

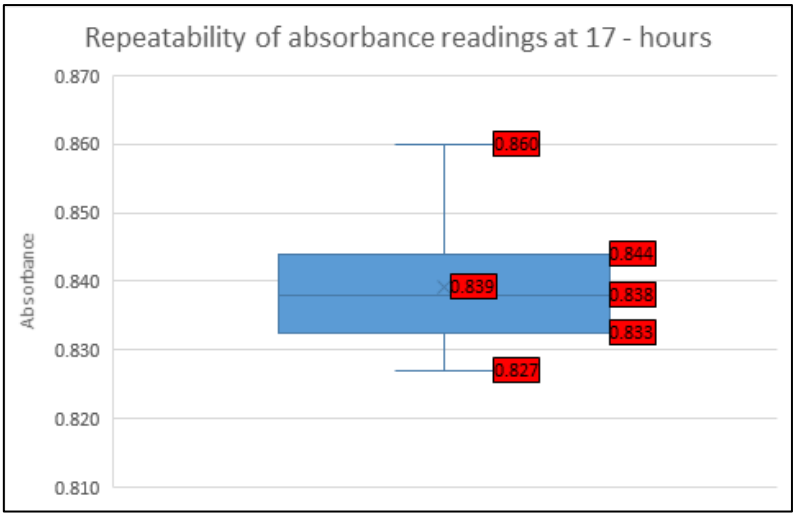


Figure A22: Box and whisker diagram for OD_{600} after 17hours

Appendix B – Microbiological procedures

Introduction to microbiological procedures

There are many general microbiological procedures that are commonly used in microbiology, but that is not common in engineering. These procedures have been written out in the next section to simplify experimental descriptions. The procedures are therefore used as building blocks to describe procedures that could seem unclear or that are often repeated.

Aseptic techniques

For all microbiology work the following procedure is followed to ensure a disinfected environment to work in, to prevent contamination as well as to ensure the safety of the researcher.

1. Put on gloves and spray gloves with ethanol.
2. Light the Bunsen burner.
3. Spray area around Bunsen burner with ethanol and wipe clean with tissue.
4. Work underneath flame of Bunsen burner to maintain aseptic conditions, as the rising warm air prevents air-borne organisms from contaminating the experimental system.
5. Spray gloves with ethanol after touching anything that might cause contamination.
6. Burn mouth of flasks and jars in flame after opening and before closing every time.
7. Put lids down with bottom facing down on ethanol cleansed surfaces under flame.
8. Heat lids and tinfoil quickly through flame before replacing.
9. Never move the lid of the petri dish more than 2cm away from the dish, preventing the dish from exposure to open air and air-borne contaminants.
10. Always work with sterile Eppendorf tubes, pipette tips, filters, and syringes.
11. Never expose sterile equipment or media to air or surfaces.

Autoclave procedure

The autoclave uses high temperature (121°C) combined with high pressure (15 psi) sustained for 30 minutes to kill all forms of micro-organisms. only autoclave-safe equipment can withstand the procedure, and caution should be exercised in autoclaving equipment.

1. Prepare the liquid or object that needs to be sterilized.
2. Tighten any lids and then loosen them slightly to prevent containers from bursting due to the build-up of pressure.
3. Close any openings with tin foil.
4. Label with autoclave tape.

5. Ensure the autoclave is ready for operation, necessary water levels etc.
6. Put in the autoclave for liquid and solid sterilization.
7. Wait for sterilization to complete and pressure to drop (2.5 hours).
8. Check that autoclave tape changed colour.

Sterilizing equipment

For equipment that is autoclave safe (glass beakers, measuring cylinders, etc.) the following procedure was used.

1. Close any open holes with tinfoil.
2. Sterilize in autoclave.

For equipment that cannot be put in the autoclave (experimental containers etc.) one of the following procedures were used.

1. Create 20% bleach solution.
2. Fill container with diluted bleach, or submerge object in diluted bleach.
3. Let it stand for 30 min.
4. Rinse repeatedly with sterile R.O. water to remove any traces of bleach.

OR

1. Spray with ethanol.
2. Wipe clean with tissue.

Preparing TSB liquid media

The following procedure was followed to prepare liquid media for bacterial growth.

1. Pour 100 ml dH_2O in 250 ml Erlenmeyer flask.
2. Add 0.3 g TSB (Tryptone Soy Broth) into the R.O. water (3 g/L, 10% the manufacturer's recommended strength).
3. Close with tin foil.
4. Label with autoclave tape.
5. Sterilise in autoclave.
6. Let it cool down to room temperature before inoculating organisms.

Preparing TSA solid media (petri dishes)

The following process was followed every time to prepare solid media in petri dishes for plating.

1. Pour 1000 ml R.O. water in 1 L glass bottle with magnetic stirrer.
2. Add 3 g TSB (Tryptic Soy Broth) (3 g/L) and 15 g Agar (15 g/L) into the distilled water.
3. Close with bottle cap, loosen cap slightly.

4. Label with autoclave tape.
5. Sterilise in autoclave.
6. Cool down on magnetic mixer immediately after the autoclave completed its cycle.
7. Wait for media to cool until it does not burn the inside of the wrist.
8. Disinfect working bench.
9. Pour media into petri dishes under aseptic conditions.
10. Fill dish ¾ of bottom full and then swirl stack of 10 dishes to cover complete plate.
11. 1000 ml TSB-Agar media makes between 60 and 70 petri dishes.
12. Leave on working bench to dry for an hour.
13. Put it upside down in 37°C incubation room for an hour to dry out.
14. Leave upside down on working bench for use.
15. Usable for between 1 and 21 days while no contamination is visible.

Sterilizing consumables

The following consumables all need to be sterilized in the autoclave before they are used in any of the sterile activities.

- Eppendorf tubes: sterilized in glass jars and stored in the same jars after sterilization.
- 200 µl pipette tips (yellow): sterilized and stored in tip boxes.
- 1000 µl pipette tips (blue): sterilized and stored in tip boxes.

Inoculating a bacteria culture

1. Prepare liquid media in Erlenmeyer flask (3.2.4).
2. Disinfect working environment.
3. Remove seal tape around petri dish with source bacteria.
4. Working aseptically, remove tin foil from flask with liquid media and heat mouth of flask to ensure no contamination.
5. Replace tinfoil loosely.
6. Sterilize inoculating loop by burning it in the flame.
7. Cool inoculating loop on agar where there is no bacterial growth.
8. Transfer a single colony from the stock bacteria culture to the liquid medium.
9. Stir liquid media in flask with inoculating loop.
10. Re-sterilize inoculating loop by burning it in flame before storing it.
11. Sterilize mouth of flask in flame and replace tin foil cap.

Incubating bacteria cultures

Bacterial cultures are maintained at optimal growth conditions for storage (solid medium) and for experimentation (liquid media).

For liquid media:

1. The liquid media is inoculated with a single colony of the Pseudomonas CT07.
2. The flask is put on a shaker at 32°C.
3. This will ensure continuous mixing while the bacteria culture is kept at the correct temperature, optimizing the distribution of oxygen through the medium and preventing the development of anaerobic layers.

For plates:

1. Plates are inverted for incubation at the appropriate temperature conditions for the corresponding time.
2. Corresponding times plates are incubated at different temperatures:
    - Room temperature – 3 days
    - 32°C – 1 day
    - 38°C – 1 day

Preparing freezer cultures

For long-term storage of bacteria, freezer cultures are prepared of a specific bacterium which can be kept for years when stored at -80°C.

1. Sterilize glycerol.
2. Inoculate Pseudomonas CT07 in liquid media.
3. Incubate bacteria under rotation at 30°C until the exponential phase is reached.
4. Sterilise working environment.
5. Working aseptically, add 750 µl glycerol to a 2-ml Eppendorf tube.
6. Working aseptically, add 750 µl bacteria containing media to the 2-ml Eppendorf tube.
7. Label Eppendorf tube with bacteria name and date.
8. Store Eppendorf tube in the freezer that maintains a temperature of -80°C.

Preparing and maintaining a bacterial working stock

For rigorous experimental consistency, a pure culture should be maintained on the bench, for easy access for inoculation.

1. Prepare petri dishes.
2. Disinfect working environment.

3. Identify a pure bacterial culture: freezer culture**, or grown liquid culture, or culture grown on plate.
4. Sterilize inoculating loop in flame.
5. Cool stick tip in liquid media or on plate to be used as source of pure culture.
6. After cooling, stir stick tip in liquid media containing bacteria or drag stick tip through bacteria culture on plate.
7. Scratch with inoculating loop on solid media in one corner of new petri dish.
8. Sterilize stick in flame.
9. Cool stick down in sterile part of solid media.
10. Pull stick through area that has been scratched and continue pulling and then scratch in a new corner of plate.
11. Sterilize stick in flame.
12. Cool stick down in sterile part of solid media.
13. Pull stick through area that has been scratched last and continue pulling and then scratch with every line being on a piece of sterile media.
14. Sterilize stick in flame.
15. Seal plate with parafilm.
16. Label plate with bacteria name and date.
17. Put plate upside down on working bench in lab.
18. Use single colonies for inoculation of media for experiments.
19. Repeat process every 3 weeks to maintain metabolic activities.

**When working with a freezer culture, all the contents of the Eppendorf tube is used to inoculate liquid media. The bacteria culture is grown two or three times, in liquid media, to ensure the culture's metabolic activities normalise.

Diluting for plating

Bacteria can be present in very high concentrations; therefore, it is necessary to dilute bacteria containing solutions before plating can be done.

1. Sterilize 1.5 ml Eppendorf tubes and saline solution (9 g/L).
2. Disinfect working environment.
3. Eppendorf tubes are prepared by adding 900 µl saline solution into every Eppendorf tube.
4. The solution to be plated is swirled a few times to mix it.
5. From the bacterial solution to be plated, 1000 µl is removed and put in an empty Eppendorf tube representing the undiluted sample.

6. The Eppendorf tube with the undiluted sample is vortexed for 3 seconds.
7. Using a new sterilised tip, 100 µl is removed from the 1000 µl and added to an Eppendorf tube containing 900 µl saline solution. This Eppendorf tube has now been diluted 1/10 or 10^{-1}.
8. The Eppendorf tube that has just received 100 µl is vortexed for 3 seconds.
9. Step 7 and step 8 are repeated until adequate number of dilutions have been done. This will differ depending on the predicted bacteria concentration.
10. The appropriate dilutions are plated.

Plating

The purpose of plating is to investigate the presence of bacteria or to quantify the bacterial concentration of a liquid, by plating a range of dilutions, typically undiluted to 10^{-7}, to ascertain at what dilution single colonies are quantifiable.

1. Prepare petri dishes with TSA (Tryptic Soy Agar) media four days prior to plating.
2. Disinfect working environment.
3. Divide petri dishes in half, labelling the plates with a marker.
4. Working aseptically, put a sterile tip on the automated pipette.
5. If plating from a large container, swirl container; if plating from an Eppendorf tube, vortex Eppendorf tube for 3 seconds.
6. Remove 110 µl of liquid, from the liquid to be plated, with the automated pipette.
7. Drop 10 drops of 10 µl each on half the plate spaced out equally.
8. Discard the tip.
9. Put a new sterile tip on the automated pipette.
10. If plating from a large container, swirl container; if plating from an Eppendorf tube, vortex Eppendorf tube for 3 seconds.
11. Remove another 110 µl of liquid, from the liquid to be plated, with the automated pipette.
12. Drop 10 drops of 10 µl each on the other half of the plate, spaced out equally.
13. Discard the tip.
14. Let plate stand for an hour.
15. Invert plate and incubate at required temperature for required time.

Plate counting and determining bacterial concentrations

The main purpose of plating is to determine the number of colony forming bacterial units (cfu) in the original liquid. Depending on the bacterial concentration, the sample will be diluted a few times before plating. From a plate with bacterial growth it is possible to distinguish different bacteria colonies if the

bacterial concentration is not too high. Generally, in microbiology and for this work, it was accepted that between 25 and 250 bacterial colonies are distinguishable on a given plate. All necessary experiments, sampling, diluting, and plating is done before this.

When the purpose is to determine bacterial concentrations:

1. After plating, inverted plated are incubated for 3 days at room temperature.
2. Identify plates that have obvious contamination, fungal or bacterial, that will influence plate counts and discard them. Note that there has been contamination. Contamination is distinguished from target culture morphologically.
3. Using a white backlight, identify the appropriate dilutions for counting single colonies (25-250 cells) and count the number of individual bacteria colonies on each half of those plates.
4. Get the average of the two halves on a single plate. If they differ significantly (half a log value) then disregard as contaminated.
5. If number of colonies counted < 25; make a note, but do not use data.
6. If number of colonies counted ≥ 250; then just note it down as ***p*** for *bacterial presence*.
7. The bacterial concentration per ml is calculated using equation 1:

$$bacterial\ concentration\ \left(\frac{cfu}{ml}\right) = average\ number\ of\ bacterial\ colonies\ on\ plate \times 10 \times 10^{number\ of\ dilution} \quad \text{(Eq. 43)}$$

When the purpose is to investigate the presence or absence of bacteria.

1. Plate directly from sample without diluting.
2. Leave plates to stand upside down for 3 days at room temperature.
3. Identify plates that have obvious contamination, fungal or bacterial, that will influence plate counts and discard them. Note that there has been contamination.
4. Using a white light, count the number of individual bacteria colonies on each half of all the plates.
5. Get the average of the two halves on a single plate. If they differ significantly (half a log value) then disregard as contaminated.
6. If number of colonies counted > 10; note bacterial presence in sample, therefore bacterial kill negative (Huang, Shih et al. 2008).
7. If number of colonies counted ≤ 10; note bacterial absence in sample, therefore bacterial kill positive.

Measuring absorbance

The optical density (OD), or absorbance, was identified as a quick method to get an indication of bacterial concentration before doing any plating or any experiments.

1. Remove 1 ml sample from a sterile TSB media solution with identical constituents to the sample that must be measured, but without any bacterial content, and put it in a 10-mm cuvette.
2. Remove 1 ml sample from the solution to be measured, and put it in a 10-mm cuvette.
3. Zero the spectrometer on 600 nm (wavelength) with the cuvette containing the TSB media as reference.
4. Cut a block of Parafilm and seal cuvette that contains bacteria.
5. Shake cuvette that is covered with tape.
6. Measure absorbance (OD_{600}).
7. Check that the reference solution still gives an OD_{600} of approximately 0.000 (± 0.010).
8. Repeat step 5 to step 7 three times to get an average OD_{600} absorbance for the sample.

Constructing a growth curve

A growth curve is done to investigate the growth pattern of a bacteria under certain conditions, by plotting absorbance and cell concentration over time, as well as absorbance vs cell concentration. The lag phase, exponential growth phase and stationary phase can be identified and used accordingly for experiments as required.

1. Prepare solid media in petri dishes.
2. Prepare 3 flasks of liquid media.
3. Inoculate the 3 flasks with unique colonies of the same bacteria.
4. Incubate the 3 flasks at 30°C.
5. Every 2 hours, from the 0 hours to 24 hours:
    - Measure absorbance of a sample from each flask.
    - Dilute a sample from each flask to 10^{-8}.
    - Plate the full dilution, from undiluted to 10^{-8}, for each flask.
6. Do plate counts after leaving plates at room temperature for 3 days.

Appendix C – Feed preparation

Raw data

Table A3: Raw data for determining OD_{600} for growth curve first round

Time	0	2	4	6	8	10	12	14	16	18	20	22	24
Reference	0.000	-0.001	0.001	-0.003	-0.002	-0.001	0.000	-0.002	0.000	-0.002	0.004	0.000	0.000
A	0.002	0.006	0.022	0.032	0.142	0.442	0.819	0.843	0.789	0.743	0.707	0.675	0.674
B	0.005	0.001	0.013	0.026	0.114	0.391	0.744	0.777	0.717	0.697	0.705	0.738	0.811
C	0.001	0.009	0.014	0.022	0.110	0.343	0.678	0.891	0.801	0.748	0.697	0.680	0.682
Average	0.003	0.005	0.016	0.027	0.122	0.392	0.747	0.837	0.769	0.729	0.703	0.698	0.722
Std Dev	0.002	0.004	0.005	0.005	0.017	0.050	0.071	0.057	0.045	0.028	0.005	0.035	0.077

Table A4: Raw data for determining OD_{600} for growth curve second round

Time		0	2	4	6	8	10	12	14	16	18	20	22	24
Reference	a	0.003	-0.001	-0.001	0.004	0.002	0.000	0.001	-0.001	0.001	-0.002	-0.001	0.000	0.004
	b	-0.004	0.000	0.000	0.000	0.004	0.006	0.001	0.005	0.000	-0.002	0.002	0.006	0.000
A	a	0.011	0.013	0.007	0.021	0.042	0.133	0.391	0.728	0.819	0.746	0.711	0.667	0.663
	b	0.014	0.018	0.007	0.018	0.036	0.128	0.379	0.705	0.805	0.739	0.710	0.657	0.654
		0.013	0.016	0.007	0.020	0.039	0.131	0.385	0.717	0.812	0.743	0.711	0.662	0.659
B	a	0.011	0.008	0.007	0.015	0.036	0.160	0.428	0.817	0.816	0.741	0.701	0.651	0.656
	b	0.016	0.008	0.007	0.016	0.035	0.159	0.418	0.804	0.804	0.736	0.693	0.649	0.649
		0.014	0.008	0.007	0.016	0.036	0.160	0.423	0.811	0.810	0.739	0.697	0.650	0.653
C	a	0.018	0.014	0.004	0.019	0.039	0.140	0.421	0.747	0.825	0.747	0.691	0.646	0.648
	b	0.020	0.005	0.008	0.016	0.035	0.138	0.412	0.757	0.822	0.729	0.689	0.649	0.635
		0.019	0.010	0.006	0.018	0.037	0.139	0.417	0.752	0.824	0.738	0.690	0.648	0.642
Average		0.015	0.011	0.007	0.018	0.037	0.143	0.408	0.760	0.815	0.740	0.699	0.653	0.651
Std. Dev		0.004	0.005	0.001	0.002	0.003	0.013	0.019	0.043	0.009	0.007	0.010	0.008	0.009

Table A5: Raw data for determining OD_{600} for third growth curve constant phase

Time		r1	r2	r3		
16	Sample	0.906	0.895	0.886	0.896	0.010
	Ref	-0.001	0.000	-0.001		
18	Sample	0.839	0.837	0.829	0.835	0.005
	Ref	-0.002	0.000	0.000		
20	Sample	0.775	0.777	0.778	0.777	0.002
	Ref	0.001	0.000	0.000		

Table A6: Raw data for determining OD_{600} for fourth growth curve constant phase

Time		r1	r2	r3		
16	Sample	0.686	0.690	0.689	0.688	0.002
	Ref	0.002	0.006	0.002		
18	Sample	0.861	0.857	0.892	0.870	0.019
	Ref	0.003	0.000	0.000		
20	Sample	0.775	0.775	0.782	0.777	0.004
	Ref	0.000	0.000	0.002		

Table A7: Raw data for determining OD_{600} for fifth growth curve constant phase

Time		r1	r2	r3		
16	Sample	0.774	0.777	0.785	0.779	0.006
	Ref	-0.004	0.002	0.001		
18	Sample	0.759	0.762	0.760	0.760	0.002
	Ref	0.001	0.003	0.000		
20	Sample	0.746	0.739	0.734	0.740	0.006
	Ref	0.000	0.005	0.010		

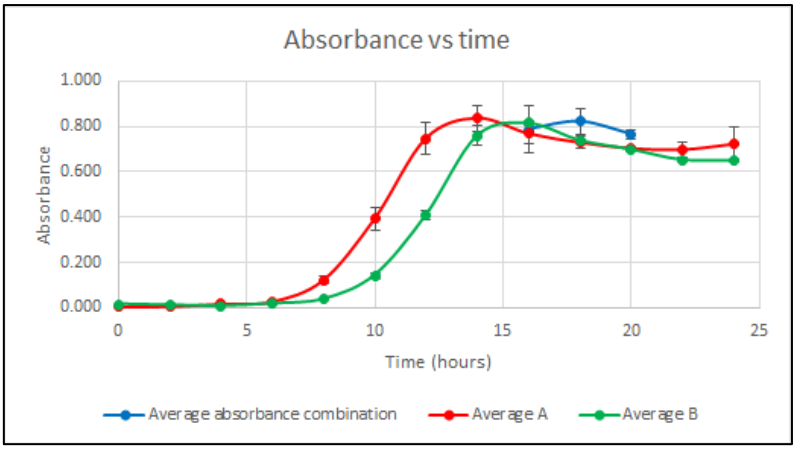


Figure A23: Absorbance for different growth curve data

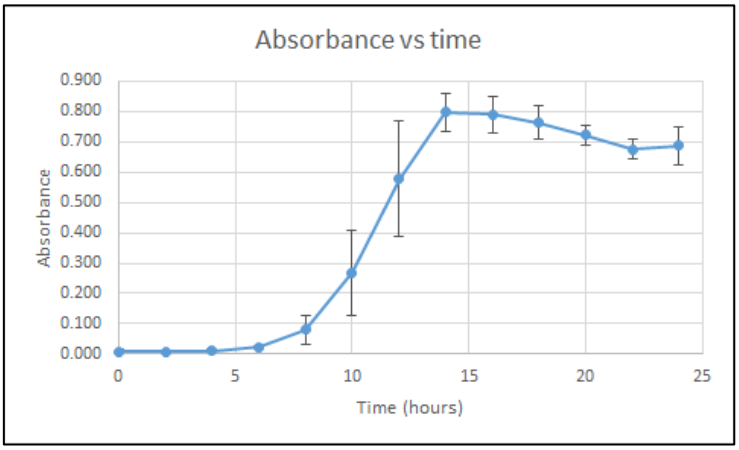


Figure A24: Absorbance for all growth curve data

Table A8: Bacterial concentrations for first growth curve

31/01/2017
A growth curve was done over a 24-hour time periode with plating and absorbance done every 2 hours for CT07. The CT07 was grown in 100 ml liquid media (TSB) at 30ºC. Every 2 hours 1 ml would be removed and put in an epi to dilute and plate and 1 ml would be put in a cuvette to measure absorbance in the spectrometre. After the cultures were returned for incubation the absorbance would be measured using a clean TSB sample as reference. The dilution and platting would then be done. This took between 30 and 40 minutes every time.

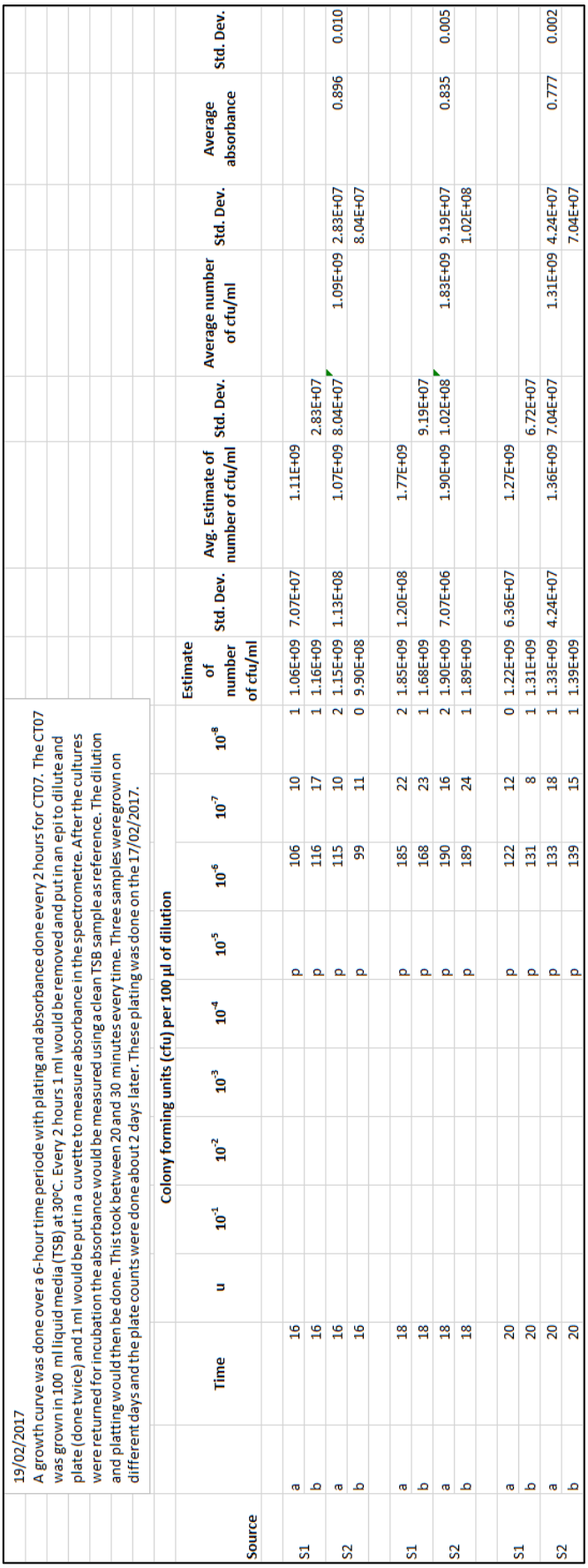
		Colony forming units (cfu) per 100 µl of dilution															
Source	Time	u	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Estimate of number of cfu/ml	Average number of cfu/ml	Absorbance	Average absorbance
A	0				p									1.00E+04		0.002	
B	0				p									1.00E+04	1.00E+04	0.005	0.003
C	0				p									1.00E+04		0.001	
A	2				p									7.00E+04		0.006	
B	2				p									7.00E+04	7.00E+04	0.001	0.005
C	2				p									7.00E+04		0.009	
A	4					p								5.00E+05		0.022	
B	4					p								5.00E+05	5.00E+05	0.013	0.016
C	4					p								5.00E+05		0.014	
A	6						p							5.00E+06		0.032	
B	6						p							5.00E+06	5.00E+06	0.026	0.027
C	6						p							5.00E+06		0.022	
A	8							p						5.00E+07		0.142	
B	8							p						5.00E+07	5.00E+07	0.114	0.122
C	8							p						5.00E+07		0.110	
A	10								p					2.00E+08		0.442	
B	10								p					2.00E+08	2.00E+08	0.391	0.392
C	10								p					2.00E+08		0.343	
A	12								p					7.00E+08		0.819	
B	12								p					7.00E+08	7.00E+08	0.744	0.747
C	12								p					7.00E+08		0.678	
A	14									p				5.00E+09		0.843	
B	14									p				5.00E+09	5.00E+09	0.777	0.837
C	14									p				5.00E+09		0.891	
A	16									p				5.00E+09		0.789	
B	16									p				5.00E+09	5.00E+09	0.717	0.769
C	16									p				5.00E+09		0.801	
A	18								p					5.00E+08		0.743	
B	18								p					5.00E+08	5.00E+08	0.697	0.729
C	18								p					5.00E+08		0.748	
A	20							p						5.00E+07		0.707	
B	20							p						5.00E+07	5.00E+07	0.705	0.703
C	20							p						5.00E+07		0.697	
A	22						p							5.00E+06		0.675	
B	22						p							5.00E+06	5.00E+06	0.738	0.698
C	22						p							5.00E+06		0.68	
A	24					p								5.00E+05		0.674	
B	24					p								5.00E+05	5.00E+05	0.811	0.722
C	24					p								5.00E+05		0.682	

Table A9: Bacterial concentrations for second growth curve

08/012/2017
A growth curve was done over a 24-hour time periode with plating and absorbance done every 2 hours for CT07. The CT07 was grown in 100 ml liquid media (TSB) at 30°C. Every 2 hours 1 ml would be removed and put in an epi to dilute and plate and 1 ml would be put in a cuvette to measure absorbance in the spectrometre. After the cultures were returned for incubation the absorbance would be measured using a clean TSB sample as reference. The dilution and platting would then be done. This took between 30 and 40 minutes every time.

				Colony forming units (cfu) per 100 µl of dilution																
Source		Time	u	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	Std. Dev.	Average number of cfu/ml	Std. Dev.	Absorbance	Average absorbance	Std. deviation absorbance	% deviation
A	a	0	p	p	28	1						2.80E+04	1.06E+04	3.55E+04				0.013			38.46%
	b	0	p	p	43	1						4.30E+04									5.49%
B	a	0	p	p	56	3						5.60E+04	1.41E+03	5.70E+04	1.17E+04	4.55E+04	1.08E+04	0.014	0.015	0.004	23.08%
	b	0	p	p	58	2						5.80E+04					1.17E+04				27.47%
C	a	0	p	p	51	1						5.10E+04	9.90E+03	4.40E+04			25.66%	0.019			12.09%
	b	0	p	p	37	2						3.70E+04									18.68%
A	a	2	p	p	32	4						3.20E+04	1.98E+04	4.60E+04				0.016			38.85%
	b	2	p	p	60	3						6.00E+04									14.65%
B	a	2	p	p	62	6						6.20E+04	6.36E+03	5.75E+04	1.07E+04	5.23E+04	5.84E+03	0.008	0.011	0.004	18.47%
	b	2	p	p	53	6						5.30E+04					1.07E+04				1.27%
C	a	2	p	p	53	10						5.30E+04	7.07E+02	5.35E+04			20.39%	0.010			1.27%
	b	2	p	p	54	11						5.40E+04									3.18%
A	a	4		p	149	19	1					1.49E+05	8.49E+03	1.43E+05				0.007			9.02%
	b	4		p	137	13	4					1.37E+05									0.24%
B	a	4		p	127	9	1					1.27E+05	1.56E+04	1.38E+05	1.06E+04	1.37E+05	7.09E+03	0.007	0.007	0.001	7.07%
	b	4		p	149	14	1					1.49E+05					1.06E+04				9.02%
C	a	4		p	124	12	2					1.24E+05	7.07E+03	1.29E+05			7.78%	0.006			9.27%
	b	4		p	134	17	2					1.34E+05									1.95%
A	a	6			p	72	7	1				7.20E+05	1.27E+05	8.10E+05				0.020			20.30%
	b	6			p	90	10	3				9.00E+05									0.37%
B	a	6			p	90	10	1				9.00E+05	2.83E+04	8.80E+05	1.13E+05	9.03E+05	1.07E+05	0.016	0.018	0.002	0.37%
	b	6			p	86	11	1				8.60E+05					1.13E+05				4.80%
C	a	6			p	104	12	0				1.04E+06	2.83E+04	1.02E+06			12.48%	0.018			15.13%
	b	6			p	100	15	1				1.00E+06									10.70%
A	a	8			p	p	62	5	0			6.20E+06	8.49E+05	6.80E+06				0.039			14.09%
	b	8			p	p	74	8	3			7.40E+06									2.54%
B	a	8			p	p	80	4	1			8.00E+06	1.06E+06	7.25E+06	7.17E+05	7.22E+06	4.01E+05	0.036	0.037	0.002	10.85%
	b	8			p	p	65	7	1			6.50E+06					7.17E+05				9.93%
C	a	8			p	p	78	3	0			7.80E+06	2.83E+05	7.60E+06			9.93%	0.037			8.08%
	b	8			p	p	74	12	0			7.40E+06									2.54%
A	a	10				p	p	28	1	0		2.80E+07	1.41E+06	2.90E+07				0.131			6.15%
	b	10				p	p	30	2	1		3.00E+07									0.56%
B	a	10				p	p	29	3	1		2.90E+07	4.24E+06	3.20E+07	3.06E+06	2.98E+07	1.89E+06	0.160	0.143	0.015	2.79%
	b	10				p	p	35	1	0		3.50E+07					3.06E+06				17.32%
C	a	10				p	p	31	7	1		3.10E+07	3.54E+06	2.85E+07			10.26%	0.139			3.91%
	b	10				p	p	26	3	1		2.60E+07									12.85%
A	a	12					p	124	6	3	0	1.24E+08	2.26E+07	1.08E+08				0.385			19.42%
	b	12					p	92	8	0	0	9.20E+07									11.40%
B	a	12					p	97	9	0	1	9.70E+07	5.66E+06	1.01E+08	1.19E+07	1.04E+08	3.69E+06	0.423	0.408	0.020	6.58%
	b	12					p	105	16	2	0	1.05E+08					1.19E+07				1.12%
C	a	12					p	110	10	1	0	1.10E+08	1.06E+07	1.03E+08			11.48%	0.417			5.94%
	b	12					p	95	16	1	0	9.50E+07									8.51%
A	a	14						187	37	3	0	1.87E+08	4.24E+06	1.84E+08				0.717			21.92%
	b	14						181	23	4	0	1.81E+08									24.43%
B	a	14						275	28	3	2	2.75E+08	1.63E+07	2.64E+08	4.48E+07	2.40E+08	4.82E+07	0.811	0.760	0.047	14.82%
	b	14						252	25	1	1	2.52E+08					4.82E+07				5.22%
C	a	14						286	38	3	1	2.86E+08	2.12E+07	2.71E+08			18.70%	0.752			19.42%
	b	14						256	39	1	0	2.56E+08									6.89%
A	a	16						p	49	4	1	4.90E+08	9.19E+07	5.55E+08				0.812			9.54%
	b	16						p	62	5	3	6.20E+08									14.46%
B	a	16						p	45	9	1	4.50E+08	7.07E+06	4.55E+08	8.47E+07	5.42E+08	8.08E+07	0.810	0.815	0.007	16.92%
	b	16						p	46	8	2	4.60E+08					8.47E+07				15.08%
C	a	16						p	59	4	2	5.90E+08	3.54E+07	6.15E+08			15.64%	0.824			8.92%
	b	16						p	64	6	1	6.40E+08									18.15%
A	a	18					p	p	64	8	1	6.40E+08	7.78E+07	5.85E+08				0.743			5.21%
	b	18					p	p	53	9	1	5.30E+08									12.88%
B	a	18					p	p	56	1	1	5.60E+08	5.66E+07	6.00E+08	5.04E+07	6.08E+08	2.84E+07	0.739	0.740	0.002	7.95%
	b	18					p	p	64	2	2	6.40E+08					5.04E+07				5.21%
C	a	18					p	p	63	4	2	6.30E+08	1.41E+07	6.40E+08			8.28%	0.738			3.56%
	b	18					p	p	65	12	1	6.50E+08									6.85%
A	a	20					p	p	p		12			Contaminated				0.711			
	b	20					p	p	p		11										
B	a	20					p	p		100	15	1.00E+09	7.78E+07	9.45E+08	7.78E+07	9.45E+08	7.78E+07	0.697	0.699	0.010	5.82%
	b	20					p	p		89	3	8.90E+08					7.78E+07				5.82%
C	a	20					p		0	0	0			Contaminated			8.23%	0.690			
	b	20					p		0	0	0										
A	a	22					p	p		80	9	8.00E+08	3.54E+07	7.75E+08				0.662			7.14%
	b	22					p	p		75	9	7.50E+08									0.45%
B	a	22					p	p		62	4	6.20E+08	1.41E+07	6.30E+08	1.24E+08	7.47E+08	1.05E+08	0.650	0.653	0.008	16.96%
	b	22					p	p		64	9	6.40E+08					1.24E+08				14.29%
C	a	22					p	p		71	10	7.10E+08	1.77E+08	8.35E+08			16.63%	0.648			4.91%
	b	22					p	p		96	7	9.60E+08									28.57%
A	a	24					p	p		90	9	9.00E+08	3.54E+07	8.75E+08				0.659			1.82%
	b	24					p	p		85	11	8.50E+08									7.27%
B	a	24					p	p		78	3	7.80E+08	1.70E+08	9.00E+08	9.05E+07	9.17E+08	5.20E+07	0.653	0.651	0.009	14.91%
	b	24					p	p		102	12	1.02E+09					9.05E+07				11.27%
C	a	24					p	p		98	8	9.80E+08	7.07E+06	9.75E+08			9.87%	0.642			6.91%
	b	24					p	p		97	8	9.70E+08									5.82%

Table A10: Bacterial concentrations for third growth curve for constant phase



19/02/2017

A growth curve was done over a 6-hour time periode with plating and absorbance done every 2 hours for CT07. The CT07 was grown in 100 ml liquid media (TSB) at 30°C. Every 2 hours 1 ml would be removed and put in an epi to dilute and plate (done twice) and 1 ml would be put in a cuvette to measure absorbance in the spectrometre. After the cultures were returned for incubation the absorbance would be measured using a clean TSB sample as reference. The dilution and platting would then be done. This took between 20 and 30 minutes every time. Three samples were grown on different days and the plate counts were done about 2 days later. These plating was done on the 17/02/2017.

Source		Time	u	Colony forming units (cfu) per 100 µl of dilution								Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	Std. Dev.	Average number of cfu/ml	Std. Dev.	Average absorbance	Std. Dev.
				10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}								
S1	a	16						p	106	10	1	1.06E+09	7.07E+07	1.11E+09					
	b	16						p	116	17	1	1.16E+09			2.83E+07				
S2	a	16						p	115	10	2	1.15E+09	1.13E+08	1.07E+09	8.04E+07	1.09E+09	2.83E+07	0.896	0.010
	b	16						p	99	11	0	9.90E+08					8.04E+07		
S1	a	18						p	185	22	2	1.85E+09	1.20E+08	1.77E+09					
	b	18						p	168	23	1	1.68E+09			9.19E+07				
S2	a	18						p	190	16	2	1.90E+09	7.07E+06	1.90E+09	1.02E+08	1.83E+09	9.19E+07	0.835	0.005
	b	18						p	189	24	1	1.89E+09					1.02E+08		
S1	a	20						p	122	12	0	1.22E+09	6.36E+07	1.27E+09					
	b	20						p	131	8	1	1.31E+09			6.72E+07				
S2	a	20						p	133	18	1	1.33E+09	4.24E+07	1.36E+09	7.04E+07	1.31E+09	4.24E+07	0.777	0.002
	b	20						p	139	15	1	1.39E+09					7.04E+07		

Table A11: Bacterial concentrations for fouth growth curve for constant phase

21/02/2017

A growth curve was done over a 6-hour time periode with plating and absorbance done every 2 hours for CT07. The CT07 was grown in 100 ml liquid media (TSB) at 30°C. Every 2 hours 1 ml would be removed and put in an epi to dilute and plate (done twice) and 1 ml would be put in a cuvette to measure absorbance in the spectrometre. After the cultures were returned for incubation the absorbance would be measured using a clean TSB sample as reference. The dilution and platting would then be done. This took between 20 and 30 minutes every time. Three samples were grown on different days and the plate counts were done about 2 days later. These plating was done on the 21/02/2017.

Source		Time	n	Colony forming units (cfu) per 100 µl of dilution								Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	Std. Dev.	Average number of cfu/ml	Std. Dev.	Average absorbance	Std. Dev.
				10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}								
S1	a	16						p	34	4	1	3.40E+08	8.49E+07	2.80E+08					
	b	16						p	22	1	0	2.20E+08			3.18E+07				
S2	a	16						p	39	4	2	3.90E+08	9.19E+07	3.25E+08	7.68E+07	3.03E+08	3.18E+07	0.688	0.002
	b	16						p	26	6	2	2.60E+08					7.68E+07		
S1	a	18						p	84	14	3	8.40E+08	7.07E+07	8.90E+08					
	b	18						p	94	7	0	9.40E+08			4.24E+07				
S2	a	18						p	101	6	2	1.01E+09	8.49E+07	9.50E+08	7.26E+07	9.20E+08	4.24E+07	0.870	0.019
	b	18						p	89	11	0	8.90E+08					7.26E+07		
												0.00E+00							
S1	a	20						p	72	6	0	7.20E+08	2.12E+07	7.35E+08					
	b	20						p	75	15	0	7.50E+08			9.19E+07				
S2	a	20						p	94	8	1	9.40E+08	1.06E+08	8.65E+08	9.76E+07	8.00E+08	1.06E+08	0.777	0.004
	b	20						p	79	12	2	7.90E+08					1.06E+08		

Table A12: Bacterial concentrations for fifith growth curve for constant phase

22/02/2017

A growth curve was done over a 6-hour time periode with plating and absorbance done every 2 hours for CT07. The CT07 was grown in 100 ml liquid media (TSB) at 30°C. Every 2 hours 1 ml would be removed and put in an epi to dilute and plate (done twice) and 1 ml would be put in a cuvette to measure absorbance in the spectrometre. After the cultures were returned for incubation the absorbance would be measured using a clean TSB sample as reference. The dilution and platting would then be done. This took between 20 and 30 minutes every time. Three samples were grown on different days and the plate counts were done about 2 days later. These plating was done on the 22/02/2017.

Source		Time	u	Colony forming units (cfu) per 100 µl of dilution								Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	Std. Dev.	Average number of cfu/ml	Std. Dev.	Average absorbance	Std. Dev.
				10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}								
S1	a		16					p	95	4	2	9.50E+08	1.41E+08	1.05E+09					
	b		16					p	115	9	2	1.15E+09			4.24E+07				
S2	a		16					p	112	12	0	1.12E+09	1.84E+08	9.90E+08	1.38E+08	1.02E+09	4.24E+07	0.779	0.006
	b		16					p	86	17	3	8.60E+08					1.38E+08		
S1	a		18					p	142	11	1	1.42E+09	1.70E+08	1.30E+09					
	b		18					p	118	15	0	1.18E+09			9.19E+07				
S2	a		18					p	122	11	1	1.22E+09	7.07E+07	1.17E+09	1.30E+08	1.24E+09	9.19E+07	0.760	0.002
	b		18					p	112	14	2	1.12E+09					1.30E+08		
S1	a		20					p	115	6	1	1.15E+09	1.27E+08	1.24E+09					
	b		20					p	133	14	1	1.33E+09			3.61E+08				
S2	a		20					p	169	16	3	1.69E+09	8.49E+07	1.75E+09	3.07E+08	1.50E+09	8.49E+07	0.740	0.006
	b		20					p	181	18	1	1.81E+09					3.07E+08		

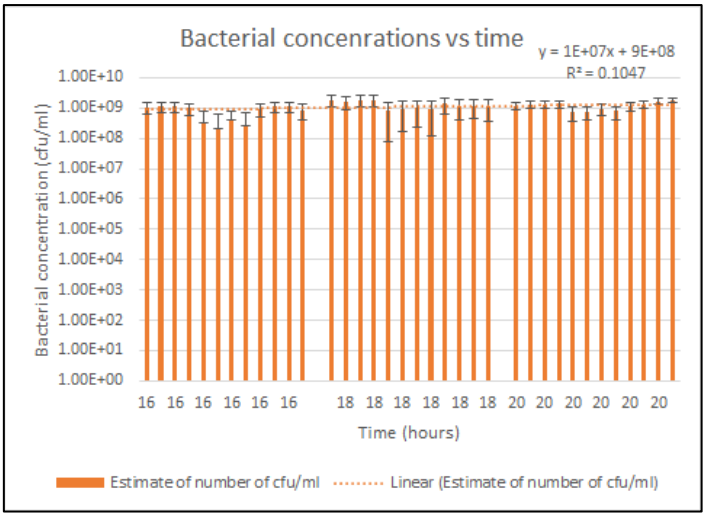


Figure A25: Bacterial concentrations for constant phase

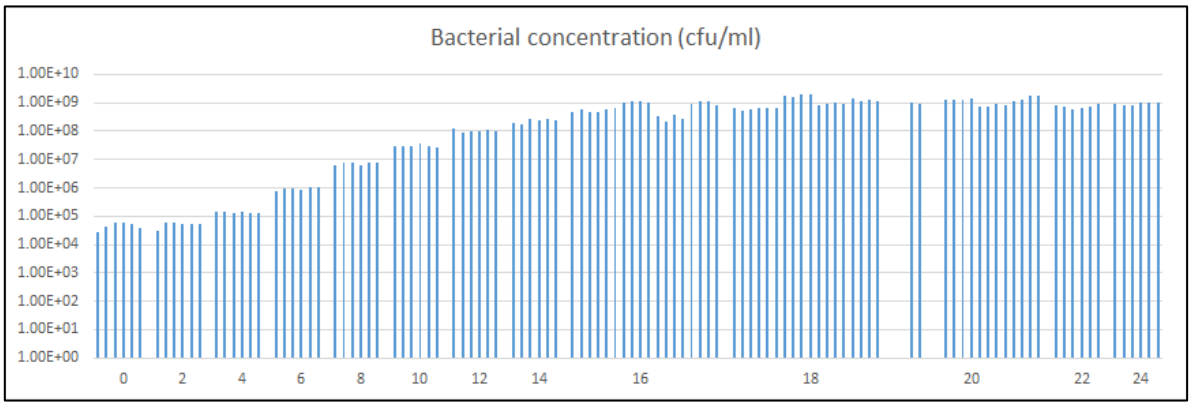


Figure A26: Bacterial concentrations for all growth curve data

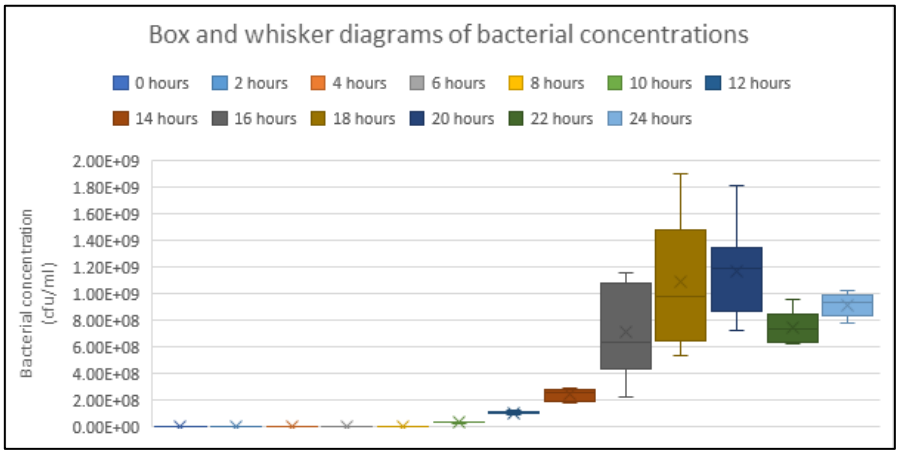


Figure A27: Data spread for growth curves on box and whisker diagram

Appendix D – BCDMH data

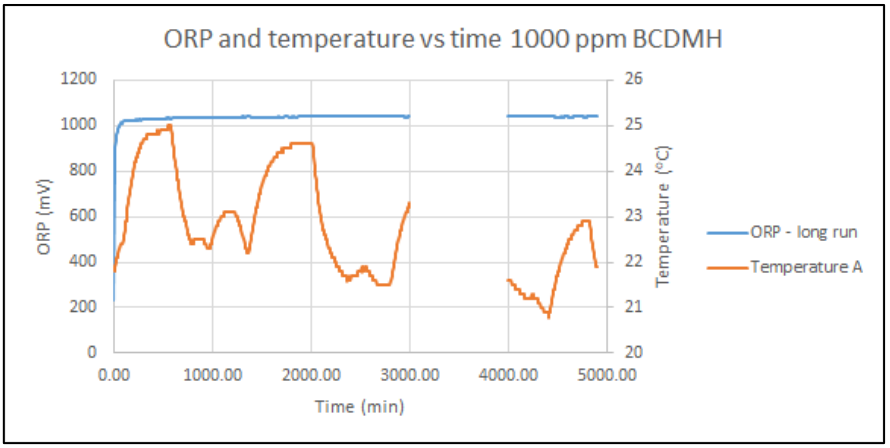


Figure A28: ORP monitor of 1000 ppm BCDMH stock solution for 80 hours

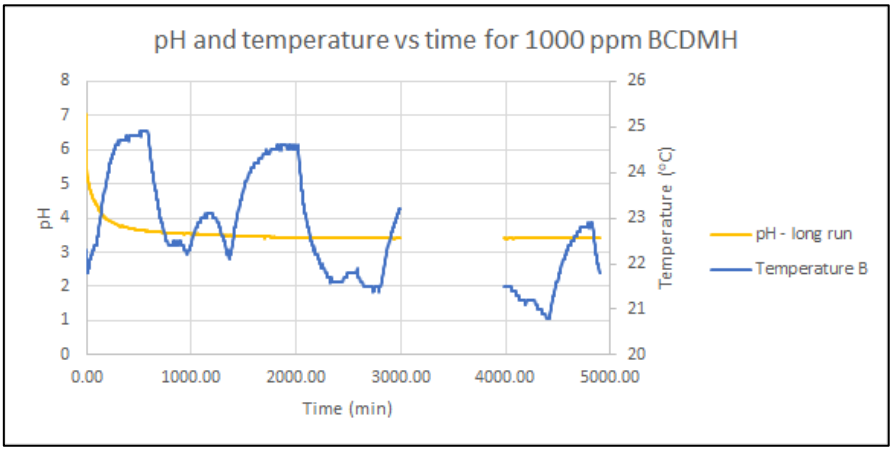


Figure A29: pH monitor of 1000 ppm BCDMH stock solution for 80 hours

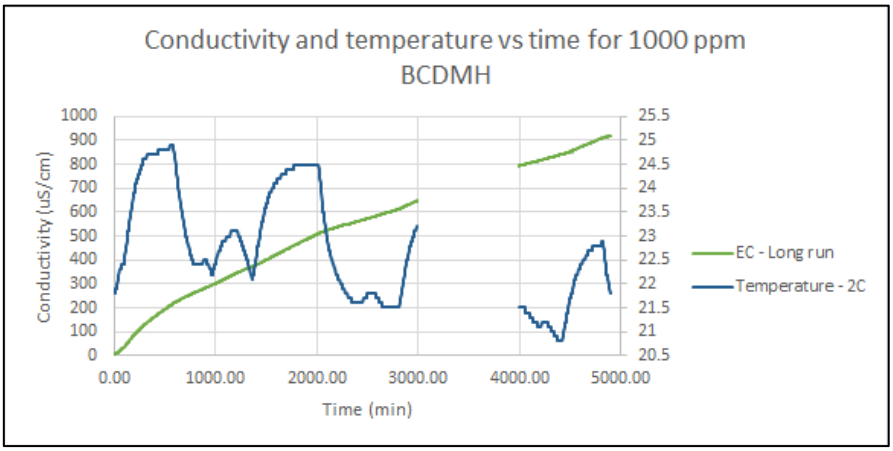


Figure A30: EC monitor of 1000 ppm BCDMH stock solution for 80 hours

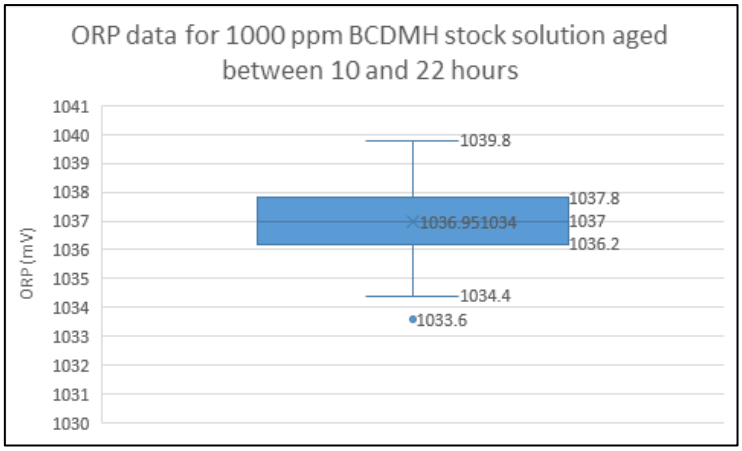


Figure A31: ORP data for BCDMH stock solution aged between 10 and 22 hours

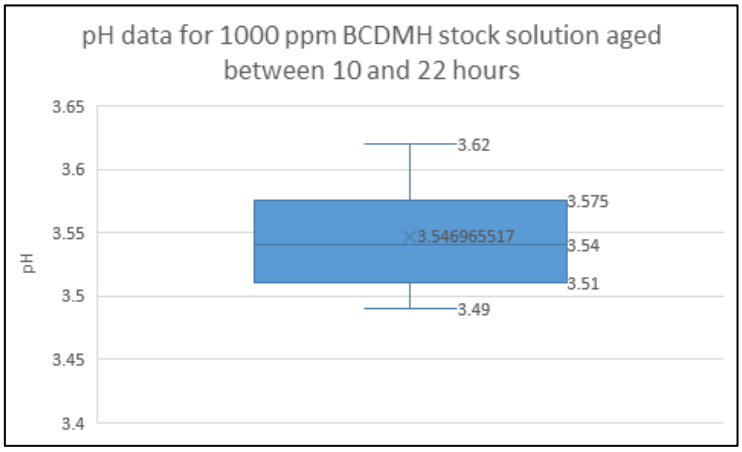


Figure A32: pH data for BCDMH stock solution aged between 10 and 22 hours

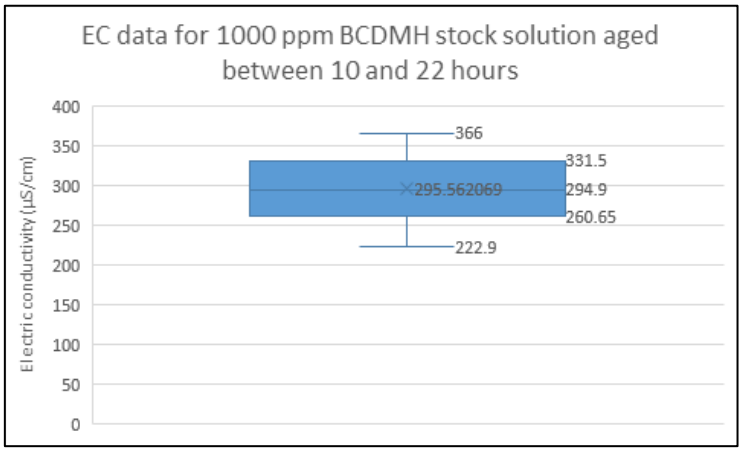


Figure A33: EC data for BCDMH stock solution aged between 10 and 22 hours

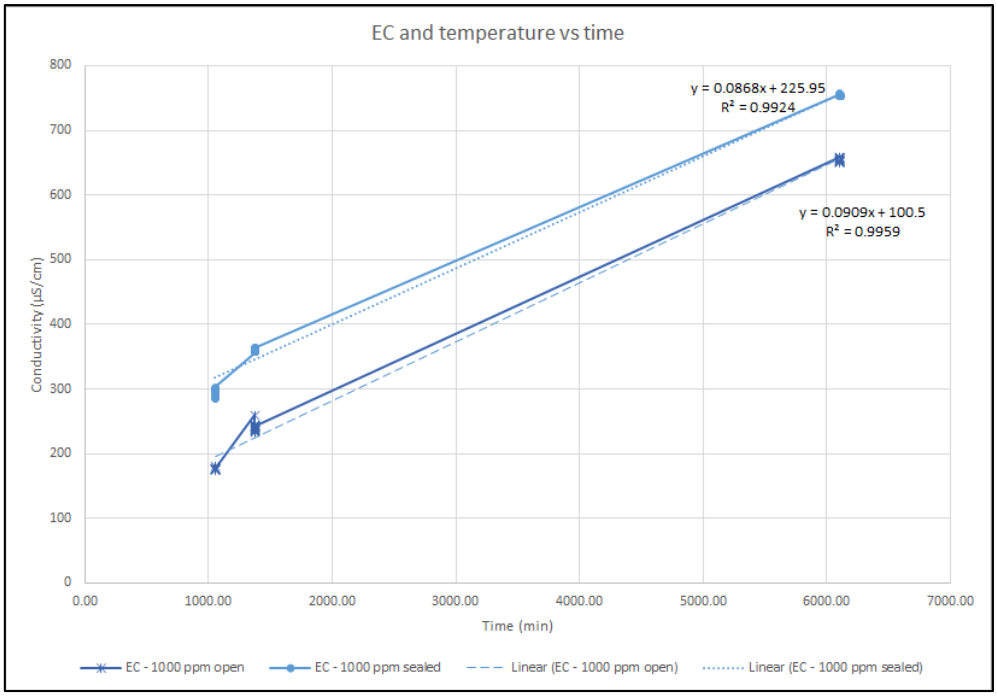


Figure A34: EC data for BCDMH stock solution sealed and unsealed

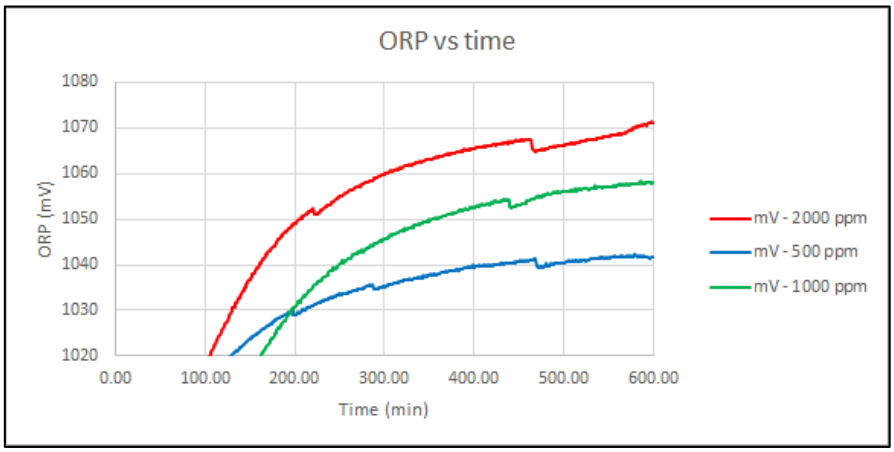


Figure A35: ORP for different BCDMH concentrations

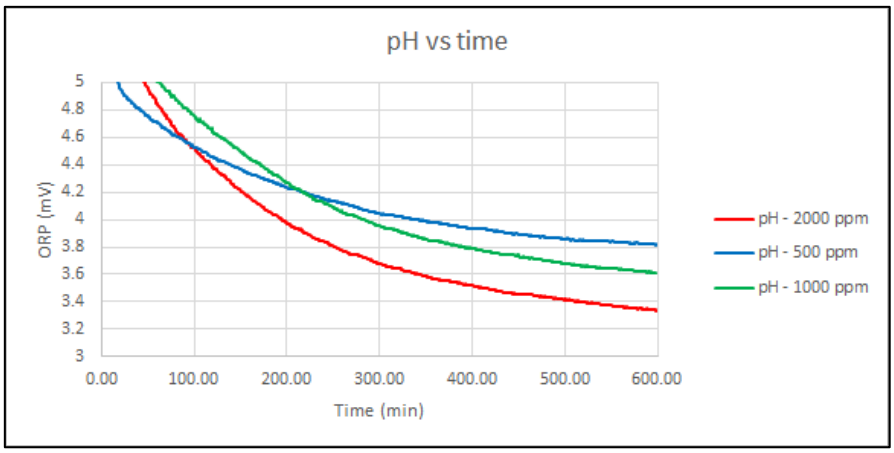


Figure A36: pH for different BCDMH concentrations

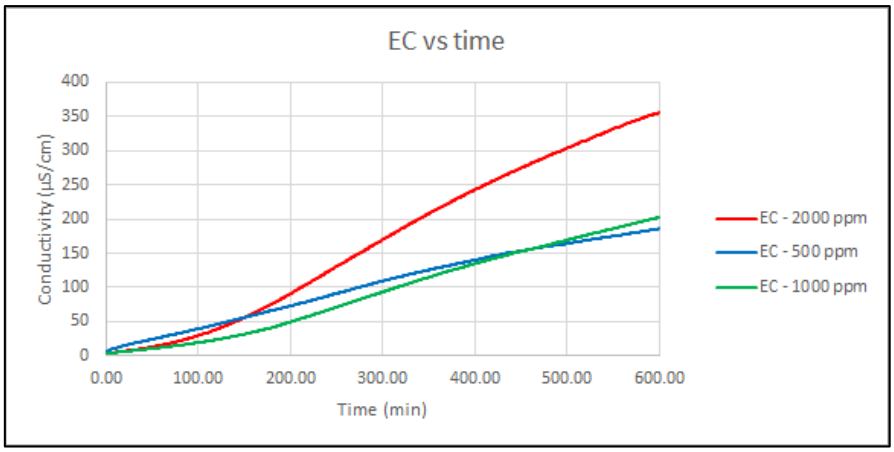


Figure A37: EC for different BCDMH concentrations

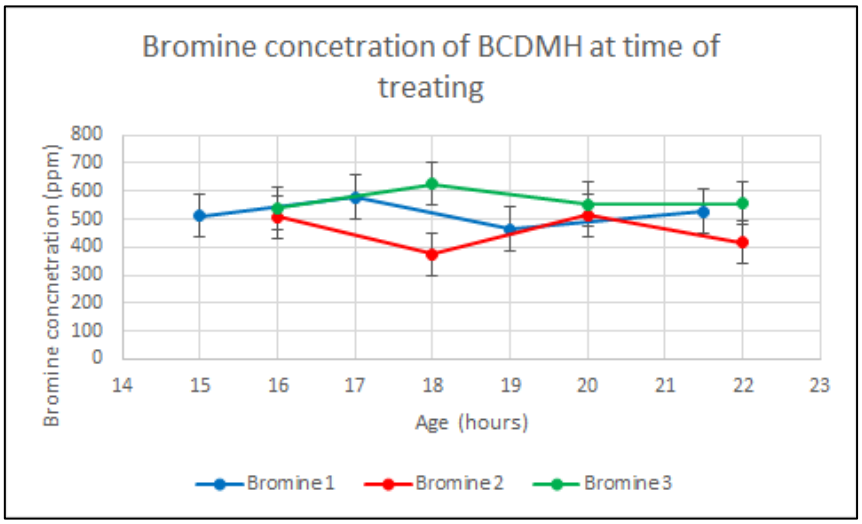


Figure A38: Bromine concentrations of BCDMH stock solution at time of treatment

Table A13: Raw data for Bromine and Free Chlorine monitoring of BCDMH stock solution

Free chlorine and bromine measurements							
Sample information				**Time:**	**Age (hours)**	**Bromine (ppm)**	**Free Chlorine (ppm)**
Water source:	R.O.			10:00	0	4	0
Volume:	1000	ml		10:10	0.166667	539	128
Mass BCDMH:	0.5	g		10:20	0.333333	552	131
Concentration:	500	ppm		10:30	0.5	572	141
Date made:	13/06/2017			11:00	1	630	147
Time made:	10:00			11:30	1.5	383	125
				12:00	2	451	143
				13:00	3	351	147
Br std. dev.	77.42			14:00	4	350	158
Cl std. dev.	23.42			16:00	6	563	128
				19:00	9	375	144
				8:00	22	413	129

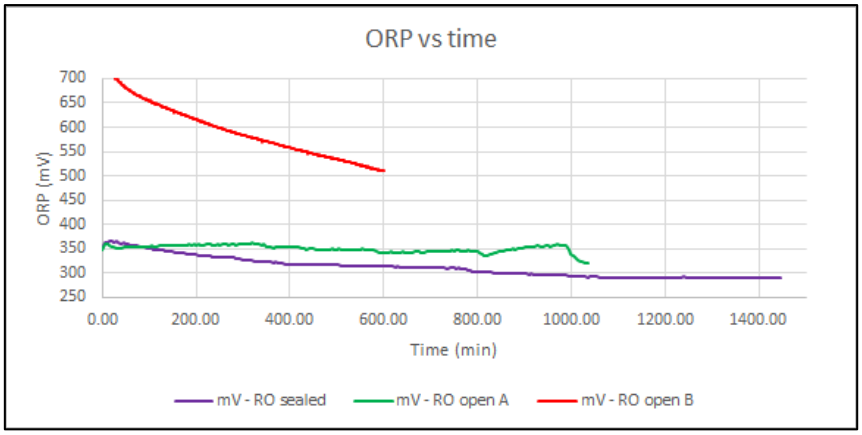


Figure A39: ORP monitor of RO water

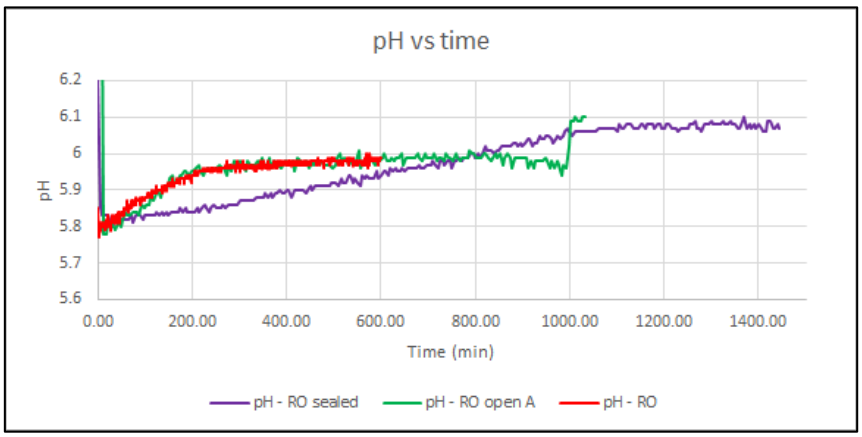


Figure A40: pH monitor of RO water

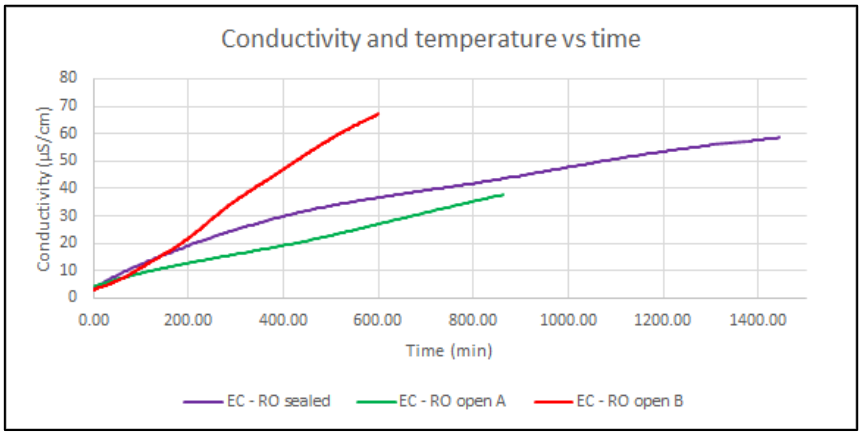


Figure A41: EC monitor of RO water

Appendix E – Ionisation data

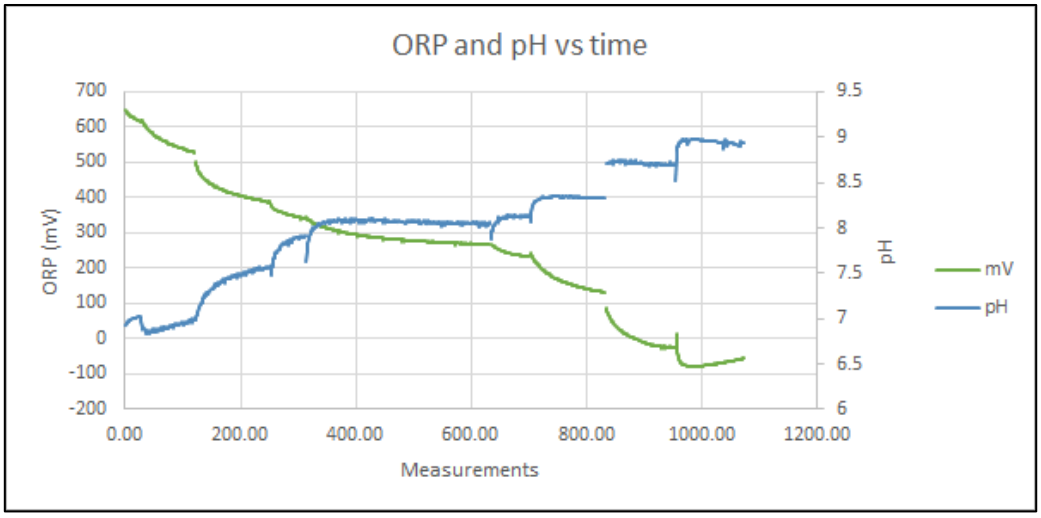


Figure A42: Ionisation effect on ORP and pH

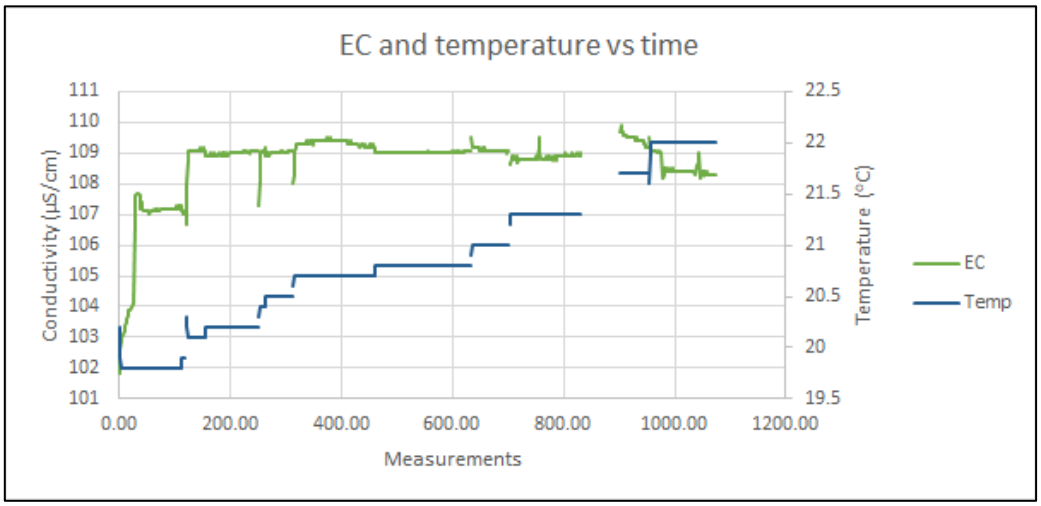


Figure A43: Ionisation effect on EC and temperature

Ionisation observations:

22/03/2017

Tap water with a voltage supply of 60 V with alloy electrodes:

- The (-) electrode (black power source) forms more gas bubbles
- The (+) electrode (red power source) forms some gas bubbles
- The + electrode losses weight
- A green-blue solution forms
- The + electrode loses more weight, - electrode seems to not really lose weight
- Current starts at 30 mA and remains relatively constant
- Closer electrodes cause higher current
- Larger surface area causes higher current
- Mixing cause a decrease in current

- When not stirred, there is a blueish layer formed on the + electrode
- This layer tries to form root like pathways to the – electrode
- Both electrodes change colour
- Higher currents cause changes in temperature
- New electrodes seem to react similar to old electrodes

30/03/2017

Multiple electrodes (silver, copper, and zinc) as anode with alloy as electrode

- Another alloy electrode was in the water and experienced a chemical reaction, blue layer forming and gas bubbles given off

31/03/2017

- With silver anode and alloy cathode
    - Water becomes whitish
    - Whitish layer form on silver anode
    - Nitric acid does not necessarily completely dissolve the silver
    - Current increases dramatically
- With parallel anode and alloy cathode
    - Cathode black layer – very thin
    - Copper brownish layer
    - Silver white (on cathode side) and black layer
    - Zinc thin dark layer

Raw data

ICP resultate: Leroi de Wet								
16-02-2017								
		Ag 328.068 {103} (Axial)	Ag 328.068 {103} (Radial)	Ag 338.289 {100} (Radial)	Cu 324.754 {104} (Radial)	Cu 327.396 {103} (Radial)	Zn 202.548 {466} (Radial)	Zn 206.200 {463} (Radial)
		Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
12	QC-ME 20ppm	20.560	20.357	19.793	20.222	19.745	20.362	20.312
13	A1	0.027	0.034	0.037	0.225	0.204	-0.004	-0.006
14	A2	0.010	0.017	0.011	0.038	0.026	-0.014	-0.014
15	A3	0.007	0.004	0.012	0.027	0.010	-0.013	-0.013
16	A4	0.004	0.008	0.008	0.016	0.003	-0.010	-0.011
17	A5	0.004	0.007	0.005	0.018	0.002	-0.007	-0.007
18	A6	0.009	0.012	0.014	0.042	0.027	-0.005	-0.005
19	A7	0.031	0.035	0.028	0.014	0.004	-0.001	0.000
20	A8	0.062	0.063	0.062	0.014	0.003	0.001	0.001
21	A9	0.119	0.085	0.082	0.018	-0.001	0.004	0.005
22	B1	0.032	0.034	0.038	0.004	-0.006	-0.019	-0.019
23	B2	0.070	0.074	0.070	1.378	1.322	1.055	1.046
24	B3	0.019	0.028	0.019	1.940	1.886	1.588	1.580
25	B4	0.010	0.014	0.016	1.269	1.224	1.984	1.980
26	B5	0.006	0.009	0.009	1.042	0.998	2.027	2.022
27	B6	0.004	0.006	0.005	0.852	0.818	1.814	1.813
28	B7	0.003	0.004	0.008	0.907	0.870	1.508	1.507
29	B8	0.003	0.004	0.004	0.869	0.837	1.041	1.036
30	C1	0.002	0.002	0.002	0.224	0.203	0.029	0.027
31	C2	0.001	0.001	0.004	0.388	0.368	0.697	0.695
32	C3	0.001	0.002	0.010	0.300	0.280	0.808	0.809
33	QC-ME 20ppm	21.436	20.704	19.739	20.747	20.524	21.054	20.971
34	C4	0.028	0.041	0.039	0.076	0.062	0.207	0.207
35	C5	0.010	0.011	0.015	0.032	0.012	0.026	0.026
36	C7	0.006	0.007	0.009	0.029	0.003	-0.002	-0.002
37	D1	0.003	0.007	0.010	0.196	0.172	0.035	0.034
38	D2	0.094	0.072	0.069	0.042	0.014	0.070	0.069
39	D3	0.864	0.841	0.795	233.313	230.327	65.797	64.041

ICP resultate: Leroi de Wet								
24-02-2017								
		Ag 328.068 {103} (Radial)	Ag 338.289 {100} (Radial)	Cu 324.754 {104} (Radial)	Cu 327.396 {103} (Radial)	Zn 202.548 {466} (Radial)	Zn 206.200 {463} (Radial)	Zn 213.856 {457} (Radial)
		Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
12	QC-ME 20ppm	20.211	19.609	19.823	19.500	19.862	19.823	19.645
13	A1	0.089	0.089	7.607	7.482	0.468	0.409	0.403
14	A2	0.022	0.019	16.924	16.610	2.383	2.256	2.209
15	A3	0.012	0.015	24.293	23.851	4.215	4.040	3.937
16	A4	0.011	0.009	28.344	27.819	5.530	5.329	5.184
17	A5	0.005	0.011	39.310	38.648	9.638	9.363	9.104
18	B1	0.013	0.008	2.386	2.333	0.308	0.290	0.286
19	B2	0.014	0.011	21.158	20.769	8.358	8.223	8.026
20	B3	0.017	0.021	32.198	31.704	13.428	13.218	12.875
21	B4	0.022	0.018	43.405	42.692	18.580	18.297	17.783
22	B5	0.021	0.024	53.827	52.834	22.944	22.591	21.871
23	B6	0.027	0.031	98.737	97.114	39.945	39.256	37.710
24	B7	0.040	0.044	278.957	276.943	118.124	115.605	106.140
25	B8	0.019	0.010	6.919	6.814	3.772	4.556	3.648
26	C1	0.020	0.020	2.368	2.322	0.285	0.268	0.262
27	C2	0.012	0.012	3.689	3.610	0.642	0.615	0.603
28	C3	0.006	0.009	4.881	4.802	1.015	0.978	0.959
29	C4	0.006	0.004	5.814	5.713	1.327	1.282	1.255
30	C5	0.002	0.002	6.703	6.567	1.607	1.555	1.520
31	C6	0.004	-0.001	7.536	7.443	1.901	1.843	1.807
32	C7	0.002	0.002	8.122	8.006	2.088	2.028	1.986
33	QC-ME 20ppm	20.210	19.595	19.957	19.720	19.985	19.941	19.793
34	D1	0.080	0.078	6.482	6.393	0.536	0.487	0.479
35	D3	0.009	0.014	134.063	133.602	39.208	38.203	36.708
36	E1	0.015	0.018	7.031	6.948	0.454	0.400	0.396
37	E4	0.004	0.009	52.675	51.806	11.785	11.399	11.100
38	E3	-0.002	0.003	102.725	101.248	25.534	24.776	24.029
	Below detection limit							
	Above calibration range							

ICP resultate: Leroi de Wet								
24-03-2017								
		Ag 328.068 {103} (Radial)	Ag 338.289 {100} (Radial)	Cu 324.754 {104} (Radial)	Cu 327.396 {103} (Radial)	Zn 202.548 {466} (Radial)	Zn 206.200 {463} (Radial)	Zn 213.856 {457} (Radial)
		Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
12	QC-ME 20ppm	20.530	19.892	20.414	19.999	20.314	20.301	20.334
13	1	0.088	0.076	0.654	0.635	0.630	0.626	0.621
14	2	0.096	0.092	0.389	0.372	0.375	0.374	0.373
15	3	0.154	0.145	0.365	0.352	0.381	0.380	0.378
16	4	0.416	0.398	0.322	0.305	0.325	0.323	0.323
17	5	2.572	2.424	0.312	0.300	0.305	0.303	0.304
18	6	15.621	14.742	0.273	0.262	0.260	0.259	0.259
19	7	15.805	14.891	0.291	0.276	0.257	0.255	0.256
20	8	0.065	0.060	0.283	0.265	0.034	0.032	0.033
21	9	0.025	0.018	10.586	10.302	0.129	0.048	0.053
22	10	0.020	0.017	20.021	19.487	0.204	0.051	0.057
23	11	0.014	0.016	0.316	0.295	0.062	0.060	0.061
24	12	0.007	0.007	0.415	0.395	10.120	10.166	10.106
25	13	0.008	0.005	0.404	0.384	15.094	15.173	15.023
26	14	0.006	0.001	0.201	0.187	0.071	0.069	0.071
27	15	0.006	0.001	7.375	7.177	0.866	0.815	0.816
28	16	0.005	0.008	12.512	12.155	1.871	1.782	1.791
29	17	0.005	0.007	0.208	0.198	0.054	0.052	0.054
30	18	0.002	-0.001	7.183	6.992	1.140	1.088	1.093
31	19	-0.002	0.004	11.884	11.542	2.109	2.025	2.039
32	20	-0.003	0.002	0.226	0.213	0.055	0.053	0.055
33	QC-ME 20ppm	20.693	19.252	20.468	19.923	19.938	19.914	20.205
34	21	0.066	0.064	7.474	7.251	1.221	1.168	1.175
35	22	0.012	0.009	12.397	12.058	2.282	2.195	2.206
36	23	0.001	0.007	0.384	0.366	0.034	0.030	0.032
37	24	0.000	0.006	3.227	3.128	0.643	0.620	0.623
38	25	-0.002	0.000	6.099	5.935	1.263	1.219	1.226
39	26	-0.004	-0.005	9.343	9.076	1.973	1.906	1.917
40	27	-0.006	-0.002	12.097	11.788	2.595	2.511	2.522
41	28	-0.008	-0.004	17.551	17.052	3.939	3.814	3.829
42	29	-0.006	-0.005	25.131	24.364	5.789	5.632	5.637
43	30	-0.009	-0.009	32.809	31.831	7.712	7.499	7.497
44	31	-0.007	-0.009	32.917	32.007	7.744	7.527	7.530
45	32	-0.008	-0.006	32.902	31.946	7.766	7.558	7.549
46	33	-0.008	-0.005	33.868	32.828	8.060	7.837	7.843
47	Water	-0.004	-0.002	0.009	0.001	0.001	0.001	0.002
		Below detection limit						

ICP resultate: Leroi de Wet								
31-03-2016								
		Ag 328.068 {103} (Radial)	Ag 338.289 {100} (Radial)	Cu 324.754 {104} (Radial)	Cu 327.396 {103} (Radial)	Zn 202.548 {466} (Radial)	Zn 206.200 {463} (Radial)	Zn 213.856 {457} (Radial)
		Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)	Y (ppm)
12	QC-ME 20ppm	20.422	19.951	20.253	19.857	19.609	19.554	19.514
13	1	0.040	0.035	-0.027	-0.029	0.141	0.142	0.140
14	2	0.015	0.010	-0.033	-0.031	0.061	0.062	0.062
15	3	0.007	0.007	-0.032	-0.035	0.027	0.027	0.027
16	4	0.004	0.006	-0.033	-0.034	0.045	0.046	0.046
17	5	0.007	0.005	-0.011	-0.012	0.064	0.065	0.065
18	6	0.004	0.007	-0.026	-0.031	0.037	0.037	0.038
19	7	0.004	0.006	-0.033	-0.034	0.063	0.065	0.065
20	8	0.005	-0.002	-0.035	-0.040	0.066	0.068	0.068
21	9	-0.002	0.001	-0.033	-0.038	0.041	0.041	0.041
22	10	0.001	0.000	0.172	0.164	0.827	0.827	0.824
23	11	0.004	0.004	0.073	0.067	0.172	0.171	0.171
24	12	0.004	0.010	-0.021	-0.024	0.027	0.027	0.028
25	13	0.147	0.140	0.787	0.786	0.240	0.233	0.234
26	14	0.068	0.063	0.202	0.195	0.062	0.061	0.061
27	15	0.051	0.049	0.105	0.100	0.037	0.037	0.037
28	16	0.007	0.006	-0.018	-0.022	0.096	0.097	0.097
29	17	0.005	0.009	-0.028	-0.034	0.051	0.051	0.052
30	18	0.021	0.014	0.335	0.329	0.085	0.082	0.082
31	19	0.019	0.014	6.350	6.396	1.594	1.543	1.539
32	20	0.008	0.014	11.191	11.278	2.959	2.870	2.862
33	QC-ME 20ppm	19.249	18.805	19.375	19.579	18.617	18.538	18.653
34	21	0.062	0.056	0.295	0.289	0.058	0.057	0.056
35	22	0.013	0.020	6.541	6.591	1.683	1.633	1.625
36	23	0.011	0.013	11.674	11.752	3.106	3.014	3.001
37	24	0.010	0.006	0.292	0.292	0.057	0.056	0.056
38	25	0.044	0.042	0.804	0.797	0.338	0.332	0.332
39	26	0.193	0.189	0.747	0.746	0.386	0.381	0.380
40	27	3.220	3.102	0.667	0.665	0.351	0.346	0.345
41	28	0.060	0.059	0.317	0.316	0.095	0.093	0.093
42	29	0.051	0.051	4.818	4.849	2.763	2.719	2.709
43	30	0.053	0.048	8.636	8.724	5.306	5.250	5.202
44	31	0.050	0.046	18.136	18.296	11.549	11.419	11.282
45	32	0.048	0.047	26.745	26.984	16.638	16.449	16.198
		Below detection limit						

2 Example of processed data

Important info

Metal		Molar mass (g/mol)	% of electrode	Electrons lost for ionisation	Percentage effective
Silver	Ag^{+}	107.868		1	100%
Copper	Cu^{2+}	63.546		2	100%
Zinc	Zn^{2+}	65.38		2	100%
Faraday	1	=	96485	Coulomb	

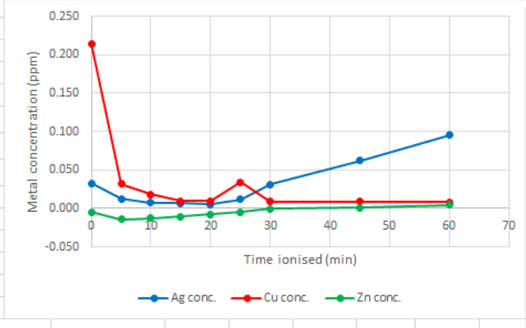
1 Amp = 1 Coulomb/sec
96 485 Coulomb = 1 Faraday

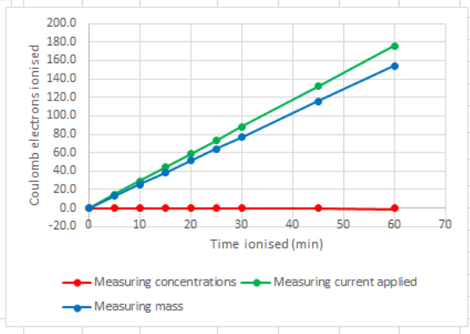
Experiment A **Date:** 2017/02/16

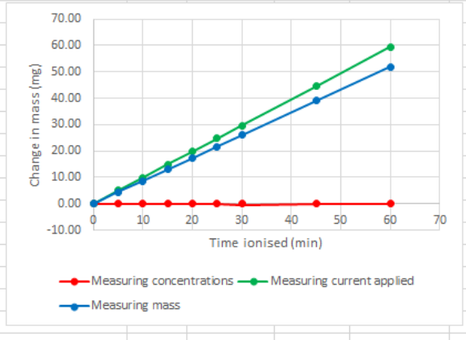
				A - Measured concentration (ppm)						B - Theoretical concentration (ppm) - calculated from applied			C - Theoretical concentration (ppm) - calcultated from change in mass				oulomb electrons ionised (			Change in mass of anode (mg			atios: A vs B concentration			atios: A vs C concentration			Change in mass ratios		
				Ag		Cu		Zn																								
Time (min)	ΔTime (min)	$I_{average}$ (mA)	Voltage (V)	Ag conc.	Std. Dev.	Cu conc.	Std. Dev.	Zn conc.	Std. Dev.	Ag conc.	Cu conc.	Zn conc.	Anode mass (g)	Ag conc.	Cu conc.	Zn conc.	Appr. A	Appr. B	Appr. C	Appr. A	Appr. B	Measured	Ag ratio	Cu ratio	Zn ratio	Ag ratio	Cu ratio	Zn ratio	A vs B	A vs C	B vs C
0	0	0	0	0.033	0.005	0.215	0.015	-0.005	0.001	0.033	0.215	-0.005	59.5420	0.033	0.215	-0.005	0.000	0.000	0.000	0.000	0.000	0.000	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	5	48.9	60	0.013	0.004	0.032	0.009	-0.014	0.000	0.776	21.262	2.966	59.5377	0.683	18.631	2.595	-0.120	14.670	12.836	-0.042	4.952	4.333	0.016	0.002	-0.005	0.019	0.002	-0.005	-0.009	-0.010	1.143
10	5	48.9	60	0.007	0.004	0.018	0.012	-0.013	0.000	1.444	40.205	5.641	59.5333	1.268	35.206	4.935	-0.140	29.340	25.672	-0.050	9.905	8.667	0.005	0.000	-0.002	0.006	0.001	-0.003	-0.005	-0.006	1.143
15	5	48.9	60	0.007	0.002	0.010	0.009	-0.011	0.000	2.046	57.254	8.047	59.5290	1.794	50.124	7.041	-0.157	44.010	38.509	-0.056	14.857	13.000	0.003	0.000	-0.001	0.004	0.000	-0.001	-0.004	-0.004	1.143
20	5	48.9	60	0.005	0.002	0.010	0.011	-0.007	0.000	2.588	72.597	10.214	59.5247	2.268	63.550	8.936	-0.169	58.680	51.345	-0.061	19.810	17.333	0.002	0.000	-0.001	0.002	0.000	-0.001	-0.003	-0.003	1.143
25	5	48.9	60	0.012	0.002	0.034	0.011	-0.005	0.000	3.075	86.407	12.163	59.5203	2.695	75.633	10.642	-0.164	73.350	64.181	-0.059	24.762	21.667	0.004	0.000	0.000	0.004	0.000	0.000	-0.002	-0.003	1.143
30	5	48.9	60	0.032	0.004	0.009	0.007	0.000	0.000	3.514	98.835	13.918	59.5160	3.079	86.508	12.177	-0.185	88.020	77.017	-0.063	29.714	26.000	0.009	0.000	0.000	0.010	0.000	0.000	-0.002	-0.002	1.143
45	15	48.9	60	0.062	0.001	0.009	0.008	0.001	0.000	5.394	152.116	21.440	59.5030	4.724	133.128	18.759	-0.191	132.030	115.526	-0.060	44.571	39.000	0.012	0.000	0.000	0.013	0.000	0.000	-0.001	-0.002	1.143
60	15	48.9	60	0.095	0.020	0.008	0.013	0.004	0.001	7.087	200.069	28.210	59.49	6.205	175.087	24.683	-0.195	176.040	154.035	-0.057	59.429	52.000	0.013	0.000	0.000	0.015	0.000	0.000	-0.001	-0.001	1.143

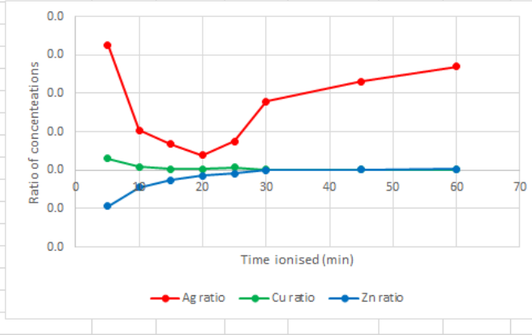
Approach A calcualtes change ins mass and electrons ionised from the measured metal concentrations
Approach B calcualtes change in mass, electrons ionised and metal concentrations from the applied current

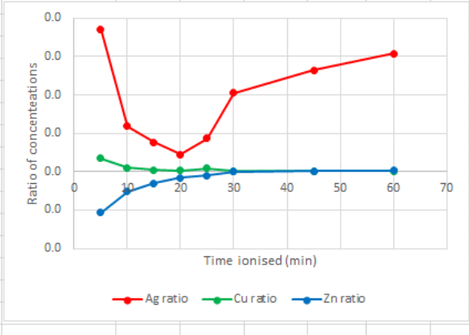
										Type	Pole
Vol:	200	ml	Source:	Tap							
I_{avg}:	48.900	mA	Stir:	600	rpm						
$A_{massbefore}$:	59.542	g	$A_{massafter}$:	59.49	g	$A_{\Delta mass}$:	52.000	mg	Electrode A:	alloy	positive
$B_{massbefore}$:	60.862	g	$B_{massafter}$:	60.858	g	$B_{\Delta mass}$:	4.000	mg	Electrode B:	alloy	negative
Samples removed and replaced:				20	ml						
Comments:											
*All samples were filtered through .22 micron filter											
Without Nitric acid											

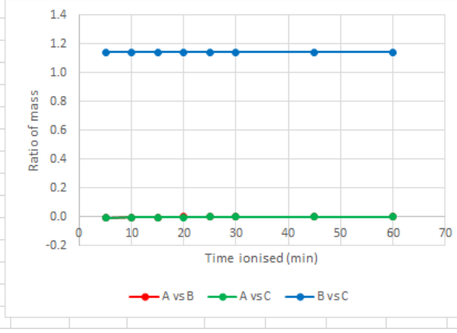












Important info

Metal		Molar mass (g/mol)	Percentage of electrode	Electrons lost for ionisation	Percentage effective
Silver	Ag^{+}	107,868		1	100%
Copper	Cu^{2+}	63,546		2	100%
Zinc	Zn^{2+}	65,38		2	100%

1 Amp = 1 Coulomb/sec
96 485 Coulomb = 1 Faraday
1 Faraday = 1 mole of electrons

Faraday	1	=	96485	Coulomb

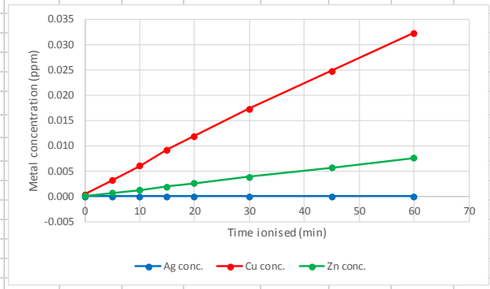
Experiment E **Date:** 2017/03/24

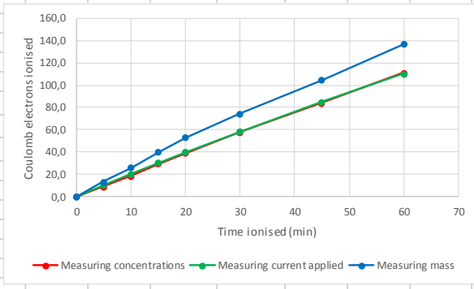
				A - Measured concentration (ppm)						B - Theoretical concentration (ppm) - calculated from applied current			C - Theoretical concentration (ppm) - calcultated from change in mass				Coulomb electrons ionised (C)			Change in mass of anode (mg)			Ratios: A vs B concentrations			Ratios: A vs C concentrations			Change in mass ratios			Electrons released ratios		
				Ag		Cu		Zn																											
Time (min)	ΔTime (min)	$I_{average}$ (mA)	Voltage (V)	Ag conc.	Std. Dev.	Cu conc.	Std. Dev.	Zn conc.	Std. Dev.	Ag conc.	Cu conc.	Zn conc.	Anode mass (g)	Ag conc.	Cu conc.	Zn conc.	Appr. A	Appr. B	Appr. C	Appr. A	Appr. B	Measured	Ag ratio	Cu ratio	Zn ratio	Ag ratio	Cu ratio	Zn ratio	A vs B	A vs C	B vs C	A vs B	A vs C	B vs C
0	0	0,00	60	0,004	0,004	0,375	0,012	0,032	0,002	0,004	0,375	0,032	59,043	0,004	0,375	0,032	0,000	0,000	0,000	0,000	0,000	0,000	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	5	34,97	60,12	0,003	0,004	3,177	0,070	0,629	0,012	0,122	3,720	0,504	59,039	0,154	4,625	0,632	9,243	10,491	13,330	3,058	3,542	4,500	0,024	0,854	1,247	0,019	0,687	0,995	0,863	0,680	0,787	0,881	0,693	0,787
10	5	33,98	60,02	-0,001	0,001	6,017	0,116	1,236	0,024	0,235	6,914	0,955	59,034	0,295	8,615	1,195	18,766	20,685	26,067	6,207	6,983	8,800	-0,004	0,870	1,294	-0,003	0,698	1,034	0,889	0,705	0,794	0,907	0,720	0,794
15	5	32,86	60,02	-0,004	0,000	9,209	0,188	1,932	0,036	0,342	9,948	1,383	59,030	0,450	13,011	1,816	29,649	30,543	40,286	9,807	10,311	13,600	-0,013	0,926	1,396	-0,010	0,708	1,064	0,951	0,721	0,758	0,971	0,736	0,758
20	5	31,72	60,06	-0,004	0,003	11,942	0,218	2,543	0,046	0,443	12,823	1,789	59,025	0,589	16,956	2,373	39,227	40,059	53,320	12,977	13,523	18,000	-0,009	0,931	1,421	-0,007	0,704	1,072	0,960	0,721	0,751	0,979	0,736	0,751
30	10	30,54	60,06	-0,006	0,003	17,301	0,353	3,861	0,068	0,642	18,458	2,585	59,018	0,819	23,480	3,294	58,012	58,383	74,648	19,196	19,709	25,200	-0,010	0,937	1,494	-0,008	0,737	1,172	0,974	0,762	0,782	0,994	0,777	0,782
45	15	29,38	60,06	-0,006	0,001	24,747	0,542	5,686	0,089	0,929	26,587	3,732	59,008	1,143	32,634	4,586	84,151	84,825	104,566	27,852	28,636	35,300	-0,006	0,931	1,524	-0,005	0,758	1,240	0,973	0,789	0,811	0,992	0,805	0,811
60	15	28,38	60,05	-0,009	0,000	32,320	0,691	7,569	0,123	1,201	34,293	4,820	58,997	1,541	43,938	6,182	111,207	110,367	136,854	36,809	37,258	46,200	-0,008	0,942	1,570	-0,006	0,736	1,224	0,988	0,797	0,806	1,008	0,813	0,806
60				-0,008	0,001	32,462	0,644	7,600	0,124																									
60				-0,007	0,001	32,424	0,676	7,624	0,123																									
60				-0,006	0,002	33,348	0,736	7,913	0,127																									

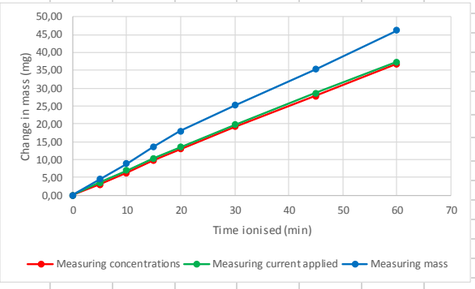
Approach A calcualtes change ins mass and electrons ionised from the measured metal concentrations
Approach B calcualtes change in mass, electrons ionised and metal concentrations from the applied current

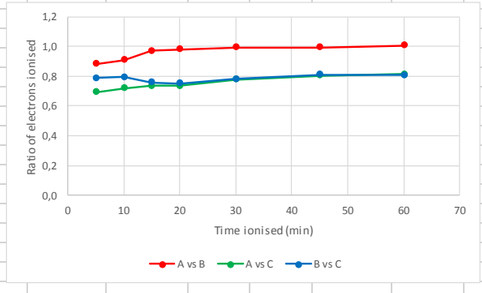
Vol:	900	ml	Source:	Tap						**Type**	**Pole**
I_{avg}:	30,658	mA	Stir:	300	rpm						
$A_{massbefore}$:	59,043	g	$A_{massafter}$:	58,997	g	$A_{\Delta mass}$:	46,200	mg	Electrode A:	alloy	positive
$B_{massbefore}$:	60,843	g	$B_{massafter}$:	60,844	g	$B_{\Delta mass}$:	-0,600	mg	Electrode B:	alloy	negative
Samples removed and replaced:				15	ml						
Comments:											

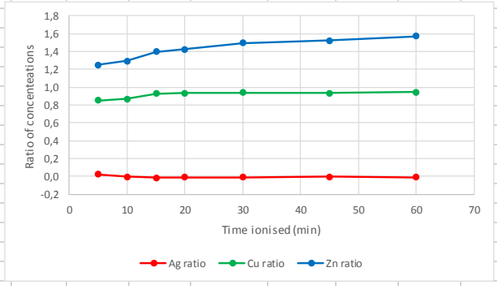
used 50 µl nitric acid per sample, repeated sample used increments of 50 µl, and added nitric acid to treated sample directly

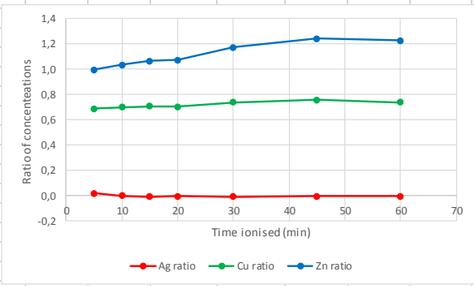


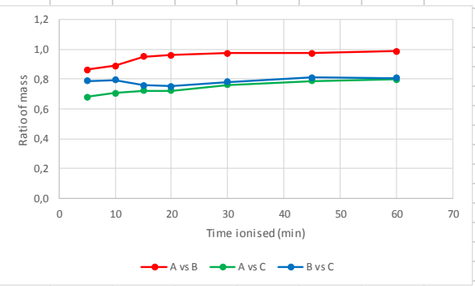












Repeatability experiments

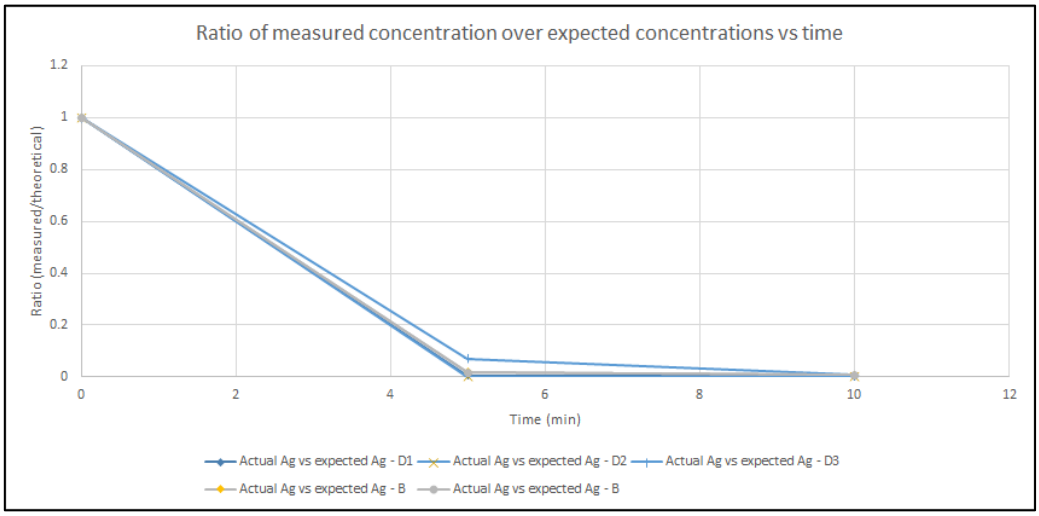


Figure A44: Ratio of measured silver concentrations compared to expected concentrations

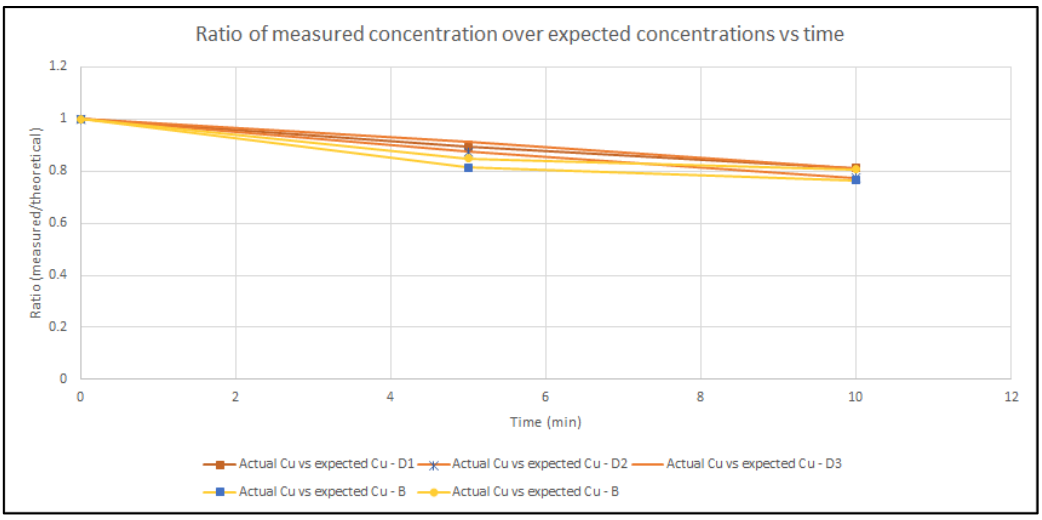


Figure A45: Ratio of measured copper concentrations compared to expected concentrations

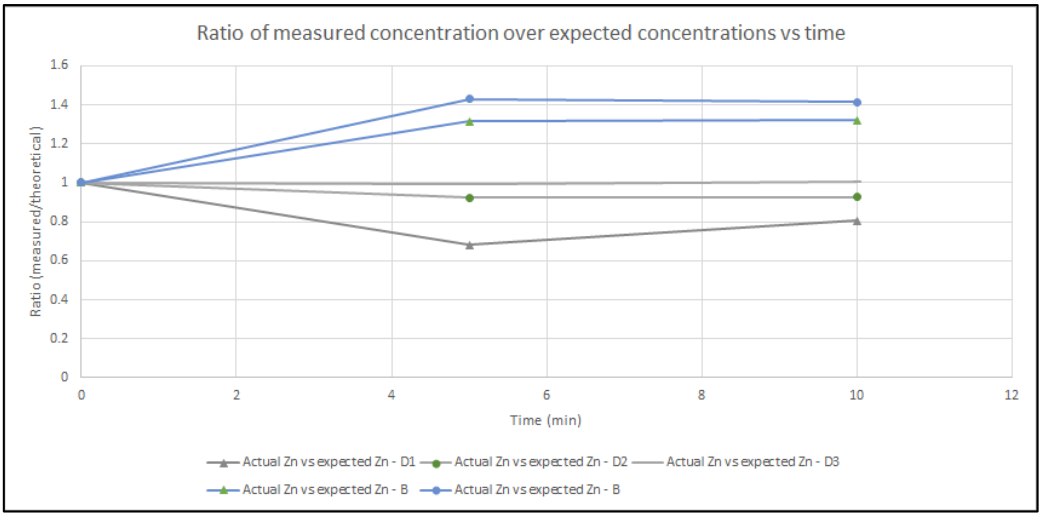


Figure A46: Ratio of measured copper concentrations compared to expected concentrations

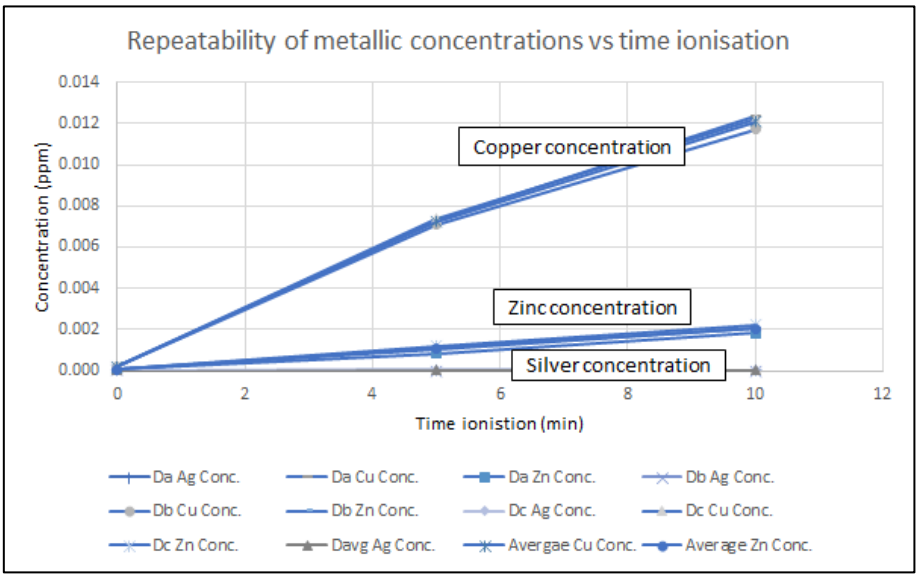


Figure A47: Repeatability of ionisation experiements

Appendix F – Treatment data

Example experimental data

Table A14: BCDMH treatment raw data experiment 1

Date:	06/04/2017												
Treatment:					**BCDMH stock solution**				**Liquid media:**	0.3 g TSB per 100 ml R.O. water			
1	100	ppm BCDMH			Concentration:		1000	ppm	**Baceria used:**	CT07 Pseudemonas			
2	40	ppm BCDMH			Volume made:		500	ml	**Water source:**	tap water			
3	10	ppm BCDMH			Date made:		03/04/2017		**Volume:**	900	ml		
					Time made:		16:00						
									Bacterial concentration		**Absorbance**		
Sample	**Inoculate date**	**Inoculate time**	**Treatment date**	**Treatment time**	**Incubation time**	**Age of BCDMH solution**	**Average absorbance**		**cfu/ml**	**Std. Dev.**	**Reading$_1$**	**Reading$_2$**	**Reading$_3$**	**Std. Dev.**
A	03/04/2017	12:00	04/04/2017	8:00	20:00	16:00	0.652		1.43E+09	9.90E+05	0.645	0.654	0.657	0.006
B	03/04/2017	13:00	04/04/2017	9:00	20:00	17:00	0.756		1.67E+09	2.34E+06	0.763	0.750	0.756	0.007
C	03/04/2017	15:00	04/04/2017	11:00	20:00	19:00	0.697		1.61E+09	2.08E+06	0.700	0.694	0.697	0.003
			Colony forming units (cfu) per 100 µl of dilution											
Source	**Treatment**		**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**After treatment undiluted**		**Estimate of number of cfu/ml**	**Std. Dev.**	**Avg. Estimate of number of cfu/ml**	**Std. Dev.**		
A	1	a	p	160	14	2	0		1.60E+07	1.05E+06	1.50E+07	9.90E+05		
		b	p	139	24	1	0		1.39E+07					
	2	a	p	136	18	3	0		1.36E+07	5.00E+04	1.36E+07			
		b	p	135	15	2	0		1.35E+07					
	3**	a	0	0	0	0	0		0.00E+00	0.00E+00	0.00E+00			
		b	0	0	0	0	0		0.00E+00					
B	1	a	p	203	22	3	0		2.03E+07	1.05E+06	1.93E+07	2.34E+06		
		b	p	182	17	1	0		1.82E+07					
	2	a	p	158	25	2	0		1.58E+07	2.50E+05	1.61E+07			
		b	p	163	13	0	0		1.63E+07					
	3	a	p	144	13	2	0		1.44E+07	3.00E+05	1.47E+07			
		b	p	150	14	1	0		1.50E+07					
B	1	a	p	200	9	1	0		2.00E+07	1.60E+06	1.84E+07	2.08E+06		
		b	p	168	16	0	0		1.68E+07					
	2	a	p	166	19	2	0		1.66E+07	1.05E+06	1.56E+07			
		b	p	145	21	2	0		1.45E+07					
	3	a	p	140	12	3	0		1.40E+07	3.50E+05	1.44E+07			
		b	p	147	18	2	0		1.47E+07					
	**experimental error													

Table B1: BCDMH treatment raw data experiment 2

Date:	21/04/2017												
Treatment:					**BCDMH stock solution**				**Liquid media:**	0.3 g TSB per 100 ml R.O. water			
1	0.0	ppm BCDMH											
2	0.5	ppm BCDMH			Concentration:		500	ppm	**Baceria used:**	CT07 Pseudemonas			
3	1.0	ppm BCDMH			Volume made:		100	ml	**Water source:**	tap water			
4	1.5	ppm BCDMH			Date made:		19/04/2017		**Volume:**	900	ml		
					Time made:		7:00						
								Bacterial concentration		**Absorbance**			
Sample	**Inoculate date**	**Inoculate time**	**Treatment date**	**Treatment time**	**Incubation time**	**Age of BCDMH solution**	**Average absorbance**	**cfu/ml**	**Std. Dev.**	**Reading$_1$**	**Reading$_2$**	**Reading$_3$**	**Std. Dev.**
A	18/04/2017	12:00	19/04/2017	8:00	20:00	1:00	0.694	1.16E+09	1.93E+06	0.696	0.690	0.697	0.004
B	18/04/2017	14:00	19/04/2017	11:00	21:00	4:00	0.645	1.30E+09	1.04E+06	0.650	0.650	0.636	0.008
C	18/04/2017	16:00	19/04/2017	14:30	22:30	7:30	0.701	1.18E+09	1.35E+06	0.712	0.698	0.693	0.010
					Colony forming units (cfu) per 100 µl of dilution								
Source	**Treatment**		**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**After treatment undiluted**	**Estimate of number of cfu/ml**	**Std. Dev.**	**Avg. Estimate of number of cfu/ml**	**Std. Dev.**		
A	1	a	p	98	14	2	p	9.80E+06	4.00E+05	1.02E+07	1.93E+06		
		b	p	106	9	2	p	1.06E+07					
	2	a	p	91	8	2	p	9.10E+06	1.00E+06	1.01E+07			
		b	p	111	13	2	p	1.11E+07					
	3	a	p	132	p	2	p	1.32E+07	1.15E+06	1.21E+07			
		b	p	109	p	2	p	1.09E+07					
	4	a	p	136	15	4	3	1.36E+07	6.00E+05	1.42E+07			
		b	p	148	13	1	2	1.48E+07					
B	1	a	p	113	15	2	p	1.13E+07	2.50E+05	1.16E+07	1.04E+06		
		b	p	118	17	1	p	1.18E+07					
	2	a	p	136	10	2	p	1.36E+07	4.00E+05	1.40E+07			
		b	p	144	7	0	p	1.44E+07					
	3	a	p	124	11	0	1	1.24E+07	4.50E+05	1.29E+07			
		b	p	133	9	2	1	1.33E+07					
	4	a	p	129	14	0	0	1.29E+07	5.00E+05	1.34E+07			
		b	p	139	10	2	0	1.39E+07					
C	1	a	p	119	10	1	p	1.19E+07	6.50E+05	1.26E+07	1.35E+06		
		b	p	132	20	0	p	1.32E+07					
	2	a	p	115	13	2	p	1.15E+07	9.00E+05	1.24E+07			
		b	p	133	13	1	p	1.33E+07					
	3	a	p	110	8	0	89	1.10E+07	1.40E+06	1.24E+07			
		b	p	138	10	3	55	1.38E+07					
	4	a	p	86	11	4	0	8.60E+06	1.15E+06	9.75E+06			
		b	p	109	10	0	0	1.09E+07					

Table A15: BCDMH treatment raw data experiment 3

Date:	06/04/2017												
Treatment:					**BCDMH stock solution**				**Liquid media:**	0.3 g TSB per 100 ml R.O. water			
1	8	ppm BCDMH			Concentration:		1000	ppm	**Baceria used:**	CT07 Pseudemonas			
2	5	ppm BCDMH			Volume made:		50	ml	**Water source:**	tap water			
3	2	ppm BCDMH			Date made:		04/04/2017		**Volume:**	900	ml		
					Time made:		16:00						
									Bacterial concentration		**Absorbance**		
Sample	**Inoculate date**	**Inoculate time**	**Treatment date**	**Treatment time**	**Incubation time**	**Age of BCDMH solution**	**Average absorbance**	**cfu/ml**	**Std. Dev.**	**Reading$_1$**	**Reading$_2$**	**Reading$_3$**	**Std. Dev.**
A	04/04/2017	11:00	05/04/2017	7:30	20:30	15:30	0.636	3.60E+09	4.53E+06	0.629	0.645	0.634	0.008
B	04/04/2017	12:00	05/04/2017	9:00	21:00	17:00	0.766	4.86E+09	3.88E+07	0.760	0.765	0.774	0.007
C	04/04/2017	14:30	05/04/2017	11:00	20:30	19:00	0.679	4.42E+09	2.35E+07	0.679	0.677	0.681	0.002

			Colony forming units (cfu) per 100 µl of dilution									
Source	**Treatment**		**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**After treatment undiluted**	**Estimate of number of cfu/ml**	**Std. Dev.**	**Avg. Estimate of number of cfu/ml**	**Std. Dev.**	
A	1	a	p	245	17	2	0	2.45E+07	1.50E+06	2.60E+07	4.53E+06	
		b	p	275	29	1	0	2.75E+07				
	2	a	p	188	12	3	0	1.88E+07	8.00E+05	1.96E+07		
		b	p	204	15	2	0	2.04E+07				
	3	a	p	p	69	0	0	6.90E+07	6.50E+06	6.25E+07		
		b	p	p	56	0	0	5.60E+07				
B	1	a	p	p	56	0	0	5.60E+07	3.70E+07	9.30E+07	3.88E+07	
		b	p	p	130	18	0	1.30E+08				
	2	a	p	p	14	4	0	1.40E+07	1.75E+07	3.15E+07		
		b	p	p	49	18	0	4.90E+07				
	3	a	p	221	p	1	p**	2.21E+07	8.00E+05	2.13E+07		
		b	p	205	p	6	0	2.05E+07				
C	1	a	p	p	35	3	0	3.50E+07	1.15E+07	4.65E+07	2.35E+07	
		b	p	p	58	0	0	5.80E+07				
	2	a	p	p	64	27	0	6.40E+07	2.50E+06	6.65E+07		
		b	p	p	69	20	0	6.90E+07				
	3	a	p	189	p	2	0	1.89E+07	8.00E+05	1.97E+07		
		b	p	205	p	1	0	2.05E+07				

Table A16: Ionisation treatment raw data experiment 1

Date:	26/04/2017											
Treatment:	Current		Time		Cu Conc. (ppm)	Zn Conc. (ppm)	Ag Conc. (ppm)	mol electrons per litre	Coulomb electrons per litre			
1	38.03	mA for	5	min	3.0056333	0.6002853	0.27010585	1.391E-04	13.422			
2	17.17	mA for	5	min	1.3570003	0.27102021	0.12194892	6.281E-05	6.060			
Liquid media:	0.3 g TSB per 100 ml R.O. water				Electrode details							
Baceria used:	CT07 Pseudemonas				% of electrode	Molar mass (g/mol)	Electrons lost for ionisation	Efficiency factor				
Treatment media:				Cu		63.546	2	0.8				
Volume:	850	ml		Zn		65.38	2	1.1				
				Ag		107.868	1	0.6				
							Bacterial concentration		Absorbance			
Sample	Inoculate date	Inoculate time	Treatment date	Treatment time	Incubation time	Average absorbance	cfu/ml	Std. Dev.	$Reading_1$	$Reading_2$	$Reading_3$	Std. Dev.
A	03/04/2017	12:00	04/04/2017	8:00	20:00	0.680	1.02E+09	0.00E+00	0.684	0.678	0.679	0.003
			Colony forming units (cfu) per 100 µl of dilution									
Source	Treatment		10^{-5}	10^{-6}	10^{-7}	10^{-8}	Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	Std. Dev.		
A		a	p	94	8	2	9.40E+08	8.00E+07	1.02E+09			
		b	p	110	17	0	1.10E+09					
			After treatment undiluted									
			0 min	20 min	40 min	60 min	80 min	100 min	120 min	140 min		
A	1	a	p	p	4	0	0	0	1	0		
		b	p	p	15	0	0	0	0	0		
	2	a	p	p	p	2	0	0	1	0		
		b	p	p	p	6	0	0	1	0		

Date:	02/06/2017												
Treatment:	Current		Time		Cu Conc. (ppm)	Zn Conc. (ppm)	Ag Conc. (ppm)	BCDMH solution added (ml)	BCDMH Conc. (ppm)	mol electrons per litre	Coulomb electrons per litre	Estimated bacterial conc. (cfu/ml)	Plate count (cfu/plate)
A1	24,9	mA for	5	min	1,859	0,371	0,167	1,98	1,100	8,602E-05	8,300	1,11E+07	2
A2	0	mA for	0	min	0,000	0,000	0,000	2,52	1,400	0,000E+00	0,000	1,11E+07	1
A3	26,92	mA for	4	min	1,608	0,321	0,144	1,98	1,100	7,440E-05	7,179	1,11E+07	1
A4	27,39	mA for	1	min	0,409	0,082	0,037	2,52	1,400	1,893E-05	1,826	1,11E+07	1
A5	27,56	mA for	2	min	0,823	0,164	0,074	2,16	1,200	3,809E-05	3,675	1,11E+07	9
A6	27,72	mA for	3	min	1,241	0,248	0,112	2,34	1,300	5,746E-05	5,544	1,11E+07	2
B1	0	mA for	0	min	0,000	0,000	0,000	2,7	1,500	0,000E+00	0,000	1,20E+07	38
B2	28,08	mA for	1	min	0,419	0,084	0,038	2,34	1,300	1,940E-05	1,872	1,20E+07	9
B3	28,11	mA for	1	min	0,420	0,084	0,038	2,52	1,400	1,942E-05	1,874	1,20E+07	4
B4	27,97	mA for	5	min	2,088	0,417	0,188	1,62	0,900	9,663E-05	9,323	1,20E+07	100
B5	28,01	mA for	2	min	0,836	0,167	0,075	2,52	1,400	3,871E-05	3,735	1,20E+07	2
B6	0	mA for	0	min	0,000	0,000	0,000	2,34	1,300	0,000E+00	0,000	1,20E+07	14
C1	28,08	mA for	4	min	1,677	0,335	0,151	2,34	1,300	7,761E-05	7,488	8,55E+06	0
C2	27,99	mA for	4	min	1,671	0,334	0,150	1,8	1,000	7,736E-05	7,464	8,55E+06	2
C3	0	mA for	0	min	0,000	0,000	0,000	2,16	1,200	0,000E+00	0,000	8,55E+06	3
C4	28,13	mA for	4	min	1,680	0,335	0,151	2,16	1,200	7,775E-05	7,501	8,55E+06	3
C5	28,15	mA for	2	min	0,840	0,168	0,076	2,34	1,300	3,890E-05	3,753	8,55E+06	1
C6	28,16	mA for	5	min	2,102	0,420	0,189	1,8	1,000	9,729E-05	9,387	8,55E+06	30

								Electrode details			
Liquid media:	0.3 g TSB per 100 ml R.O. water		BCDMH stock solution					% of electrode	Molar mass (g/mol)	Electrons lost for ionisation	Efficiency factor
Baceria used:	CT07 Pseudemonas		Concentration:	500	ppm	Cu		85%	63,546	2	0,8
Treatment media:	sterilized tap water		Volume made:	100	ml	Zn		12%	65,38	2	1,1
Volume:	900 ml		Date made:	01/06/2017		Ag		3%	107,868	1	0,6
Volume bacteria added:	9 ml		Time made:	16:00							
BCDMH contact time:	5 min										

								Bacterial concentration		Absorbance			
Sample	Inoculate date	Inoculate time	Treatment date	Treatment time	Incubation time (h)	Age of BCDMH solution (h)	Average absorbance	cfu/ml	Std. Dev.	$Reading_1$	$Reading_2$	$Reading_3$	Std. Dev.
A	01/06/2017	12:00	02/06/2017	8:00	20:00	16:00	0,594	1,11E+09	6,50E+07	0,588	0,597	0,597	0,005
B	01/06/2017	14:00	02/06/2017	10:00	20:00	18:00	0,717	1,20E+09	4,00E+07	0,719	0,706	0,725	0,010
C	01/06/2017	16:00	02/06/2017	12:00	20:00	20:00	0,680	8,55E+08	6,50E+07	0,677	0,682	0,681	0,003

	Colony forming units (cfu) per 100 µl of dilution								
Source		10^{-5}	10^{-6}	10^{-7}	10^{-8}	Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml	
A	a	p	104	13	1	1,04E+09	6,50E+07	1,11E+09	
	b	p	117	10	1	1,17E+09			
B	a	p	116	11	1	1,16E+09	4,00E+07	1,20E+09	
	b	p	124	13	2	1,24E+09			
C	a	p	92	3	3	9,20E+08	6,50E+07	8,55E+08	
	b	p	79	13	1	7,90E+08			

Treatment:	BCDMH Conc. (ppm)	mol electrons per litre	End Free bromine (ppm)	End ORP (mv)	End pH	End conductivity (µS/cm)	Plate count (cfu/plate)
A1	1,100	8,602E-05	0,520	283	7,54	122,4	2
A2	1,400	0,000E+00	0,730	350	7,29	117,4	1
A3	1,100	7,440E-05	0,570	257	8,11	115,2	1
A4	1,400	1,893E-05	0,310	272	7,89	114,7	1
A5	1,200	3,809E-05	0,380	255	8	114,8	9
A6	1,300	5,746E-05	0,550	260	7,78	114,7	2
B1	1,500	0,000E+00	0,500	330	7,33	114,8	38
B2	1,300	1,940E-05	0,230	309	7,44	116,3	9
B3	1,400	1,942E-05	0,650	303	7,5	116,4	4
B4	0,900	9,663E-05	0,210	231	8,11	115,2	100
B5	1,400	3,871E-05	0,250	260	7,62	115,6	2
B6	1,300	0,000E+00	0,670	307	7,42	115,2	14
C1	1,300	7,761E-05	0,520	253	7,78	114,7	0
C2	1,000	7,736E-05	0,350	207	8,19	115,3	2
C3	1,200	0,000E+00	0,220	261	7,73	115,7	3
C4	1,200	7,775E-05	0,540	217	8,32	113,7	3
C5	1,300	3,890E-05	0,540	218	8,14	113,7	1
C6	1,000	9,729E-05	0,490	187	8,39	114	30

Date:	31/05/2017												
Treatment:	Current		Time		Cu Conc. (ppm)	Zn Conc. (ppm)	Ag Conc. (ppm)	BCDMH solution added (ml)	BCDMH Conc. (ppm)	mol electrons per litre	Coulomb electrons per litre	Estimated bacterial conc. (cfu/ml)	Plate count (cfu/plate)
A1	24,28	mA for	3	min	1,087	0,217	0,098	1,62	0,900	5,033E-05	4,856	9,55E+06	120
A2	25,98	mA for	2	min	0,776	0,155	0,070	1,8	1,000	3,590E-05	3,464	9,55E+06	160
A3	0	mA for	0	min	0,000	0,000	0,000	1,8	1,000	0,000E+00	0,000	9,55E+06	p
A4	26,98	mA for	1	min	0,403	0,080	0,036	1,8	1,000	1,864E-05	1,799	9,55E+06	180
A5	27,11	mA for	2	min	0,809	0,162	0,073	2,16	1,200	3,746E-05	3,615	9,55E+06	3
A6	27,17	mA for	5	min	2,028	0,405	0,182	1,8	1,000	9,387E-05	9,057	9,55E+06	34
B1	0	mA for	0	min	0,000	0,000	0,000	1,62	0,900	0,000E+00	0,000	1,01E+07	p
B2	27,59	mA for	1	min	0,412	0,082	0,037	2,16	1,200	1,906E-05	1,839	1,01E+07	2
B3	27,55	mA for	2	min	0,823	0,164	0,074	1,62	0,900	3,807E-05	3,673	1,01E+07	p
B4	27,64	mA for	1	min	0,413	0,082	0,037	1,62	0,900	1,910E-05	1,843	1,01E+07	p
B5	27,71	mA for	1	min	0,414	0,083	0,037	1,98	1,100	1,915E-05	1,847	1,01E+07	28
B6	27,7	mA for	3	min	1,241	0,248	0,111	1,8	1,000	5,742E-05	5,540	1,01E+07	p
C1	27,73	mA for	4	min	1,656	0,331	0,149	2,16	1,200	7,664E-05	7,395	1,07E+07	1
C2	27,68	mA for	2	min	0,826	0,165	0,074	1,98	1,100	3,825E-05	3,691	1,07E+07	8
C3	25,72	mA for	3	min	1,152	0,230	0,104	2,16	1,200	5,331E-05	5,144	1,07E+07	6
C4	0	mA for	0	min	0,000	0,000	0,000	1,98	1,100	0,000E+00	0,000	1,07E+07	31
C5	27,11	mA for	3	min	1,214	0,242	0,109	1,98	1,100	5,620E-05	5,422	1,07E+07	55
C6	27,34	mA for	4	min	1,633	0,326	0,147	1,62	0,900	7,556E-05	7,291	1,07E+07	p

								Electrode details			
Liquid media:	0.3 g TSB per 100 ml R.O. water		BCDMH stock solution					% of electrode	Molar mass (g/mol)	Electrons lost for ionisation	Efficiency factor
Baceria used:	CT07 Pseudemonas		Concentration:	500	ppm		Cu	85%	63,546	2	0,8
Treatment media:	sterilized tap water		Volume made:	100	ml		Zn	12%	65,38	2	1,1
Volume:	900 ml		Date made:	30/05/2017			Ag	3%	107,868	1	0,6
Volume bacteria added:	9 ml		Time made:	16:00							
BCDMH contact time:	5 min										

								Bacterial concentration		Absorbance			
Sample	Inoculate date	Inoculate time	Treatment date	Treatment time	Incubation time (h)	Age of BCDMH solution (h)	Average absorbance	cfu/ml	Std. Dev.	$Reading_1$	$Reading_2$	$Reading_3$	Std. Dev.
A	30/05/2017	12:00	31/05/2017	8:00	20:00	16:00	0,610	9,55E+08	6,50E+07	0,602	0,615	0,613	0,007
B	30/05/2017	14:00	31/05/2017	10:00	20:00	18:00	0,647	1,01E+09	5,00E+06	0,642	0,648	0,650	0,004
C	30/05/2017	16:00	31/05/2017	12:00	20:00	20:00	0,580	1,07E+09	3,50E+07	0,570	0,586	0,585	0,009

			Colony forming units (cfu) per 100 µl of dilution						
Source			10^{-5}	10^{-6}	10^{-7}	10^{-8}	Estimate of number of cfu/ml	Std. Dev.	Avg. Estimate of number of cfu/ml
A	a	p	89	7	1		8,90E+08	6,50E+07	9,55E+08
	b	p	102	11	0		1,02E+09		
B	a	p	101	11	2		1,01E+09	5,00E+06	1,01E+09
	b	p	100	9	0		1,00E+09		
C	a	p	103	10	1		1,03E+09	3,50E+07	1,07E+09
	b	p	110	8	0		1,10E+09		

Treatment:	BCDMH Conc. (ppm)	mol electrons per litre	End Free bromine (ppm)	End ORP (mv)	End pH	End conductivity (µS/cm)	Plate count (cfu/plate)
A1	0,900	5,033E-05	0,430	381	7,62	121,7	120
A2	1,000	3,590E-05	0,300	405	7,55	122,3	160
A3	1,000	0,000E+00	0,350	415	7,39	121,7	p
A4	1,000	1,864E-05	0,270	406	7,43	121,8	180
A5	1,200	3,746E-05	0,450	401	7,49	122,8	3
A6	1,000	9,387E-05	0,230	378	7,97	126	34
B1	0,900	0,000E+00	0,400	475	7,36	130,2	p
B2	1,200	1,906E-05	0,500	472	7,43	128,2	2
B3	0,900	3,807E-05	0,190	434	7,83	118,6	p
B4	0,900	1,910E-05	0,300	440	7,72	117,4	p
B5	1,100	1,915E-05	0,440	447	7,56	125,1	28
B6	1,000	5,742E-05		394	8,24	125,3	p
C1	1,200	7,664E-05	0,300	413	8,07	125,6	1
C2	1,100	3,825E-05	0,330	360	8	130,6	8
C3	1,200	5,331E-05	0,360	339	8,32	125,1	6
C4	1,100	0,000E+00	0,340	382	7,86	125,6	31
C5	1,100	5,620E-05	0,150	337	8,18	124,4	55
C6	0,900	7,556E-05		305	8,33	123,9	p

Monitoring experimental data

The ORP, pH and EC monitors monitored the samples continuously. Table A17 to table A19 are examples of the output data stored as .csv files.

Table A17: Example of ORP monitor output

Meter: HANNA INSTRUMENTS edge							
Lot: MVLOT001.CSV							
Meter ID: 0001							
Meter SN: C0238003							
Meter Firmware: 1.08							
Interval: 5 seconds							
Probe Model: HI36180							
Probe SN: 400000							
Probe Firmware: 1.01							
GLP Date: 2017-03-02							
GLP Time: 09:13							
Offset: -222.2mV							
	#Rec.	Date	Time	mV	unit	Temperat	unit
	1	2017/06/02	10:09:07	263.5	mV	22.3	°C
	2	2017/06/02	10:09:12	242.9	mV	26.3	°C
	3	2017/06/02	10:09:17	229.9	mV	27.6	°C
	4	2017/06/02	10:09:22	227.4	mV	28.1	°C
	5	2017/06/02	10:09:27	223.6	mV	28.4	°C
	6	2017/06/02	10:09:32	222.8	mV	28.5	°C
	7	2017/06/02	10:09:37	222.8	mV	28.6	°C
	8	2017/06/02	10:09:42	221.2	mV	28.6	°C
	9	2017/06/02	10:09:47	219.6	mV	28.7	°C
	10	2017/06/02	10:09:52	220.6	mV	28.7	°C

Table A18: Example of pH monitor output

Meter: HANNA INSTRUMENTS edge									
Lot: PHLOT001.CSV									
Meter ID: 0003									
Meter SN: C03081F9									
Meter Firmware: 1.08									
Interval: 5 seconds									
Probe Model: HI11310									
Probe SN: 037055									
Probe Firmware: 1.04									
GLP Date: 2017-05-29									
GLP Time: 12:14									
Calibration Buffers(pH): 7.01									
Offset: -7.5mV									
Average Slope: 100.0%									
Condition: ----									
Response: ----									
	#Rec.	Date	Time	pH	unit	mV	unit	Temperat	unit
	1	2017/06/02	10:09:07	7.41	pH	-31.4	mV	20.9	°C
	2	2017/06/02	10:09:12	7.52	pH	-38.2	mV	23.3	°C
	3	2017/06/02	10:09:17	7.61	pH	-43.9	mV	25.2	°C
	4	2017/06/02	10:09:22	7.68	pH	-48.2	mV	26.2	°C
	5	2017/06/02	10:09:27	7.69	pH	-48.8	mV	27.1	°C
	6	2017/06/02	10:09:32	7.76	pH	-52.9	mV	27.5	°C
	7	2017/06/02	10:09:37	7.85	pH	-58.5	mV	27.8	°C
	8	2017/06/02	10:09:42	7.89	pH	-60.6	mV	28.1	°C
	9	2017/06/02	10:09:47	7.91	pH	-61.8	mV	28.3	°C
	10	2017/06/02	10:09:52	7.94	pH	-63.8	mV	28.4	°C

Table A19: Example of EC monitor output

Meter: HANNA INSTRUMENTS edge							
Lot: ECLOT001.CSV							
Meter ID: 0002							
Meter SN: C0128203							
Meter Firmware: 1.07							
Interval: 5 seconds							
Probe Model: HI763100							
Probe SN: 106523							
Probe Firmware: 1.04							
GLP Date: 2017-05-29							
GLP Time: 12:18							
GLP Standard: 0uS/cm 84uS/cm							
Offset: 0.00uS/cm							
C.F.(cm-1): 1.030							
T.Coef(%/°C): 1.90							
T.Ref(°C): 25							
	#Rec.	Date	Time	EC	unit	Temperat	unit
	1	2015/01/05	5:09:07	98.8	uS/cm	25.1	°C
	2	2015/01/05	5:09:12	96	uS/cm	27.8	°C
	3	2015/01/05	5:09:17	95	uS/cm	28.6	°C
	4	2015/01/05	5:09:22	94.7	uS/cm	28.9	°C
	5	2015/01/05	5:09:27	94.6	uS/cm	28.9	°C
	6	2015/01/05	5:09:32	94.6	uS/cm	28.9	°C
	7	2015/01/05	5:09:37	94.6	uS/cm	28.9	°C
	8	2015/01/05	5:09:42	94.5	uS/cm	28.9	°C
	9	2015/01/05	5:09:47	94.7	uS/cm	28.8	°C
	10	2015/01/05	5:09:52	94.6	uS/cm	28.8	°C

The data from the monitors were processed slightly to create graphs to represent all the data. Figure A48 to figure A50 are examples of the graphs representing the monitored data.

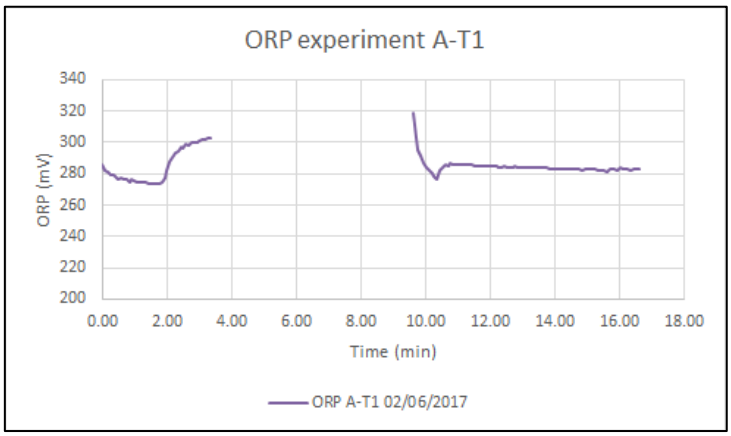


Figure A48: ORP monitor of experiment A-T1 on 02/06/2017

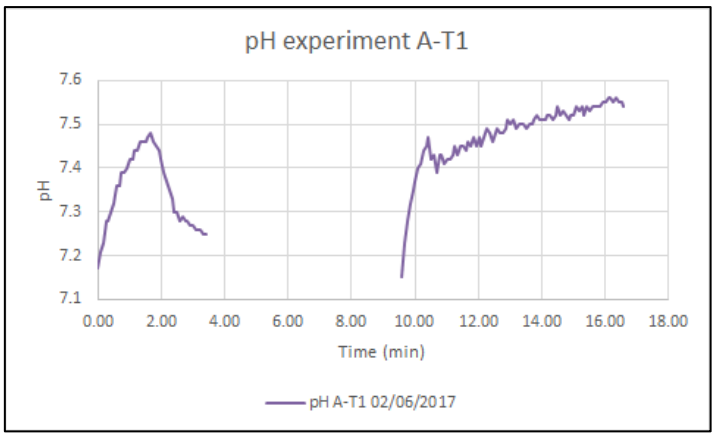


Figure A49: pH monitor of experiment A-T1 on 02/06/2017

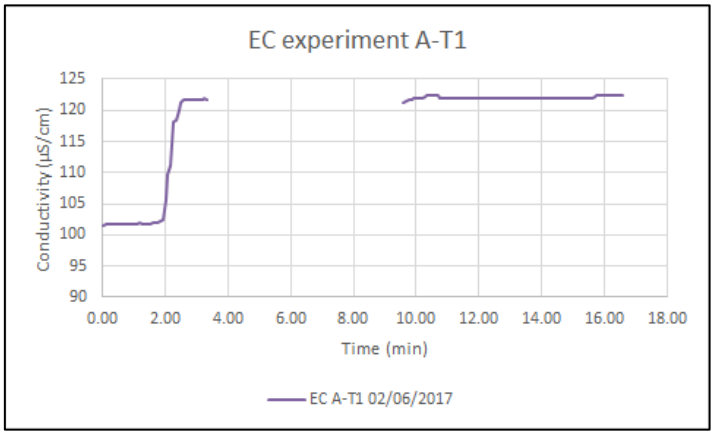


Figure A50: EC monitor of experiment A-T1 on 02/06/2017

Appendix G – Results

Example of results processed and statisica ouput

Table A20: Probability models

x_1	x_2	(P)		
1,723964	0	0,95		
1,236518	7	0,95		
0,487446				
28,27%				
	$x_2 = 0$	$x_2 = 7$		
(π)	x_1	x_1	Δx_1	$\%\Delta x_1$
0,1191	**0,981**	**0,981**	0,000	0,00%
0,2	**1,074**	**1,013**	0,061	5,64%
0,3	**1,154**	**1,041**	0,114	9,85%
0,4	**1,221**	**1,064**	0,157	12,88%
0,5	**1,282**	**1,084**	0,197	15,39%
0,6	**1,343**	**1,105**	0,237	17,67%
0,7	**1,409**	**1,128**	0,281	19,93%
0,8	**1,490**	**1,156**	0,334	22,41%
0,9	**1,612**	**1,198**	0,414	25,67%
0,95	**1,724**	**1,237**	0,487	28,27%
0,96	**1,759**	**1,249**	0,510	29,02%
0,965	**1,780**	**1,256**	0,524	29,45%
0,97	**1,804**	**1,264**	0,540	29,93%
0,975	**1,832**	**1,274**	0,558	30,48%
0,98	**1,866**	**1,285**	0,581	31,12%
0,985	**1,910**	**1,301**	0,610	31,92%
0,99	**1,972**	**1,322**	0,650	32,97%
0,9999	**2,665**	**1,560**	1,105	41,46%
1	**6,469**	**2,868**	3,602	55,67%

Table A21: Statistica data sets

All data sets

All data sets	Disinfection - Model building results					
	Distribution : BINOMIAL					
	Link function: LOGIT Modeled probability that Disinfection = 1					
	Var. 1	**Var. 2**	**Var. 3**	**Degr. of Freedom**	**Likelihd Score**	**p**
1	BCDMH concentration	Coulomb electrons	Interaction	3	23,96082	0,000025
2	Coulomb electrons	Interaction		2	15,18341	0,000505
3	BCDMH concentration	Interaction		2	12,73334	0,001718
4	BCDMH concentration	Coulomb electrons		2	10,96188	0,004165
5	BCDMH concentration			1	10,90561	0,000959
6	Coulomb electrons			1	4,11124	0,042599
7	Interaction			1	0,17474	0,675934

Effect	Disinfection - Test of all effects (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1		
	Degr. of Freedom	Wald Stat.	p
Intercept	1	12,10586655	0,000502634
BCDMH concentration	1	10,33467663	0,00130554
Coulomb electrons	1	3,619791172	0,05709604
Interaction	1	4,374754586	0,036475082

Effect	Disinfection - Parameter estimates (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1									
	Level of Effect	Column	Estimate	Standard Error	Wald Stat.	Lower CL 95,0%	Upper CL 95,0%	p		
Intercept		1	-8,533924745	2,452736351	12,10586655	-13,3412	-3,72665	0,000503	0,000197	
BCDMH concentration		2	6,658117175	2,071109153	10,33467663	2,598818	10,71742	0,001306	779,0827	1,94607
Coulomb electrons		3	-1,781578805	0,936404041	3,619791172	-3,6169	0,053739	0,057096	0,168372	0,168372
Interaction		4	1,815764219	0,868125686	4,374754586	0,114269	3,517259	0,036475	6,145771	1,199106
Scale			1	0		1	1			

Effect	Disinfection - Odds Ratios (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1					
	Level of Effect	Column	Odds Ratio	Lower CL 95,0%	Upper CL 95,0%	p
Intercept		1				
BCDMH concentration		2	779,0826787	13,44783097	45135,14643	0,001306
Coulomb electrons		3	0,168372111	0,026865912	1,055209568	0,057096
Interaction		4	6,145771099	1,121053805	33,69196217	0,036475

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	Disinfection - Statistics of goodness of fit (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1 (Analysis sample		
	Df	Stat.	Stat/Df
Deviance	127	81,83514065	0,644371186
Scaled Deviance	127	81,83514065	0,644371186
Pearson Chi²	127	84,82337607	0,667900599
Scaled P. Chi²	127	84,82337607	0,667900599
AIC		89,83514065	
AICC		90,15260097	
BIC		101,3359299	
Cox-Snell R²		0,515653389	
Nagelkerke R²		0,696199458	
Loglikelihood		-40,91757032	

	Testing Global Null Hypothesis: BETA=0 (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1 (Analysis sample)		
	Chi-Square	Df	p
Likelihood Ratio	94,96903823	3	0
Score	23,96082056	3	2,54549E-05
Wald	26,30751186	3	8,22304E-06

Response	Disinfection - Goodness of Fit: Hosmer-Lemeshow Test (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Hosmer Lemeshow = -119,6051, p value = 1										
	Group1	Group2	Group3	Group4	Group5	Group6	Group7	Group8	Group9	Group10	Row Tot.
0: Observed	13	13	13	13	13	13	13	13	13	14,0	131
Expected	-467	-436	-557	-517	-650	-596	-741	-675	-833	14,0	
1: Observed	0	0	0	0	0	0	0	0	0	0,0	0
Expected	480	449	570	530	663	609	754	688	846	0,0	
All Groups	13	13	13	13	13	13	13	13	13	14,0	131

	Classification of cases (alldatapoints) Odds ratio: 38,727273 Log odds ratio: 3,656544		
	Predicted: 1	Predicted: 0	Percent correct
Observed: 1	42	11	79,245283
Observed: 0	7	71	91,025641

	Disinfection - Estimated correlation matrix of estimates (BCDMH and metal 2) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection			
	Intercept	BCDMH concentratic	Coulomb electrons	Interaction
Intercept	1	-0,986765985	-0,469382694	0,427488881
BCDMH concentration	-0,986765985	1	0,485512542	-0,455846062
Coulomb electrons	-0,469382694	0,485512542	1	-0,993632352
Interaction	0,427488881	-0,455846062	-0,993632352	1

Data without extreme values

All data sets	Disinfection - Model building results				
	Distribution : BINOMIAL				
	Link function: LOGIT Modeled probability that Disinfection = 1				
	Var. 1	**Var. 2**	**Degr. of Freedom**	**Likelihd Score**	**p**
1	BCDMH concentration	Interaction	2	39,12113	0,000000
2	BCDMH concentration		1	35,56419	0,000000
3	Interaction		1	0,18629	0,666024

Effect	Disinfection - Test of all effects (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1		
	Degr. of Freedom	Wald Stat.	p
Intercept	1	27,38962689	1,66321E-07
BCDMH concentration	1	24,93817265	5,91986E-07
Interaction	1	3,98035392	0,04603389

Effect	Disinfection - Parameter estimates (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1							
	Level of Effect	Column	Estimate	Standard Error	Wald Stat.	Lower CL 95,0%	Upper CL 95,0%	p
Intercept		1	-12,06890738	2,306082798	27,38962689	-16,5887	-7,54907	1,66321E-07
BCDMH concentration		2	9,740327435	1,950478839	24,93817265	5,917459	13,5632	5,91986E-07
Interaction		3	0,182082267	0,091265535	3,98035392	0,003205	0,360959	0,04603389
Scale			1	0		1	1	

Effect	Disinfection - Odds Ratios (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1				
	Level of Effect	Column	Odds Ratio	Lower CL 95,0%	Upper CL 95,0%	p
Intercept		1				
BCDMH concentration		2	16989,1033	371,4666757	777000,064	5,92E-07
Interaction		3	1,199712886	1,003210246	1,434705253	0,046034

	Disinfection - Statistics of goodness of fit (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1 (Analysis sample)		
	Df	Stat.	Stat/Df
Deviance	101	86,2323657	0,853785799
Scaled Deviance	101	86,2323657	0,853785799
Pearson Chi²	101	91,5077446	0,906017273
Scaled P. Chi²	101	91,5077446	0,906017273
AIC		92,2323657	
AICC		92,4723657	
BIC		100,1655384	
Cox-Snell R²		0,383532506	
Nagelkerke R²		0,524694697	
Loglikelihood		-43,11618285	

	Testing Global Null Hypothesis: BETA=0 (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1 (Analysis sample)		
	Chi-Square	Df	p
Likelihood Ratio	50,30996724	2	1,1894E-11
Score	39,12112748	2	3,19856E-09
Wald	25,47584014	2	2,93759E-06

Response	Disinfection - Goodness of Fit: Hosmer-Lemeshow Test (Spreadsheet1) Distribution : BINOMIAL, Link function: LOGIT Hosmer Lemeshow = 1.7318, p value = 0.98816										
	Group1	Group2	Group3	Group4	Group5	Group6	Group7	Group8	Group9	Group10	Row Tot.
0: Observed	11	17	8	8	8	6	4	3	1	0	66
Expected	10,73989485	16,57146642	8,737635951	8,080580146	7,359419857	6,387422	4,829077	2,314172625	0,949649	0,030682	
1: Observed	0	1	2	2	2	4	7	7	9	4	38
Expected	0,260105149	1,428533575	1,262364049	1,919419854	2,640580143	3,612578	6,170923	7,685827375	9,050351	3,969318	
All Groups	11	18	10	10	10	10	11	10	10	4	104

	Classification of cases (alldatapoints) Odds ratio: 20.688312 Log odds ratio: 3,029569		
	Predicted: 1	Predicted: 0	Percent correct
Observed: 1	27	11	71,0526316
Observed: 0	7	59	89,3939394

	Disinfection - Estimated correlation matrix of estimates (BCDMH and metal 2) Distribution : BINOMIAL, Link function: LOGIT Modeled probability that Disinfection = 1		
	Intercept	BCDMH concentratic	Interaction
Intercept	1	-0,982178443	-0,395160237
BCDMH concentration	-0,982178443	1	0,257634223
Interaction	-0,395160237	0,257634223	1